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Variations of intestinal calcium absorption in adult frogs (*Rana esculenta*). Effect of lysine

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Summary. Intestinal calcium absorption was investigated in an adult frog (*Rana esculenta*) by injecting a $CaCl_2$ solution containing ^{45}Ca into the lumen. The ^{45}Ca absorption coefficient in the proximal loop was higher than in the distal loop, only when the $CaCl_2$ solution was left for 4 h. This coefficient increased both in the proximal and distal loops when a 4-h treatment was substituted for a 1-h treatment. The coefficient increased in the whole intestine during the first 2 h of treatment (1 h : 21 % ; 2 h : 55 %) and remained stable afterwards in our experimental conditions. The intestinal calcium absorption increase occurred early in the presence of L-lysine (100 mM), since the coefficient already reached its maximum value (52 %) after a 1-h treatment.

Introduction.

In mammals a correlation has been established between the degree of phosphorylation of the membrane proteins of intestinal microvilli (brush border) and the permeability of this membrane to calcium (Dupuis *et al.*, 1984). This protein phosphorylation could make the microvillus membrane more permeable to calcium, as after addition of phosphorylable compounds (*e.g.* carbohydrates, creatine, L-lysine) to the diet. On the contrary the decrease in intestinal calcium absorption triggered by phosphates could be related to a larger phosphorylation of the membrane proteins. The main phosphorylable protein in the membrane of intestinal microvilli seems to be alkaline phosphatase (Dupuis *et al.*, 1981 ; Fournier and Dupuis, 1982).

In amphibians, the protein phosphorylation pattern of the intestinal microvillus membrane has recently been investigated (El Maraghi-Ater *et al.*, 1986). Time-dependent protein phosphorylation increases more slowly in adult frogs (*Rana esculenta*) than in mammals and is significantly inhibited by the addition of L-lysine to the incubation medium. As in mammals the main phosphorylable protein seems to be alkaline phosphatase. The calcium metabolism of meta-

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morphosed amphibians undergoes important modifications (Dacke, 1979 ; Hourdry and Beaumont, 1985). From the juvenile period on, the intestine becomes the main organ concerned with calcium absorption. The absorbed calcium is deposited as carbonate in the « chalky » and endolymphatic sacs ; it acquires a preponderant role in the regulation of plasma pH in juvenile and adult amphibians, whose pulmonary respiration is too weak to control plasma pH, as in mammals and birds. Moreover precluding the production of a more rigid skeleton compatible with jumping in a terrestrial environment, calcium from the « chalky » and endolymphatic sacs is transferred to the bones being remodelled, where it is deposited as phosphates and carbonate. In the present paper we studied the intestinal calcium permeability with or without L-lysine. The results suggest the existence of a relationship between intestinal calcium absorption and the degree of phosphorylation of microvillus membrane proteins.

Materials and methods.

Calcium absorption *in vivo* was investigated in 54 adult frogs (*Rana esculenta*), according to the method of Dupuis *et al.* (1980). The animals were purchased from Lessieux (France) and weighed between 40 and 45 g. They were fed minced meat. After an overnight fast, each animal was anesthetized with ether and its intestine isolated *in situ* by tying off. The intestine was total from pylorus to rectal pouch or isolated into two loops, *i.e.* a proximal loop from pylorus to mid-intestine and a distal loop from mid-intestine to rectal pouch. Each animal was given 0.3 ml of a 10 mM $CaCl_2$ solution (containing 3.7 kBq ^{45}Ca/ml) either with or without L-lysine at a 100 mM concentration, by injection into the ligated part. After injection the intestine was replaced in the abdominal cavity immediately closed by sewing. The frogs were sacrificed 1, 2 or 4 h following the injection.

Each total intestine or loop, including the wall and content, was then ashed in an oven (600 °C for 12 h). The ashes were solubilized in a 1 % HNO_3 heated solution. The ^{45}Ca absorption coefficient was calculated according to the formula

$$\frac{\text{injected } ^{45}Ca - \text{residual } ^{45}Ca}{\text{injected } ^{45}Ca} \times 100$$

in which the residual ^{45}Ca is equivalent to the total intestine or loop ^{45}Ca. Student's t-test was used for statistical analysis.

The radioactivity was also measured in the plasma, after centrifugation of blood samples and in shin-bone ashes solubilized in HNO_3 (concentrated acid then 4 % solution).

Results.

Intestinal calcium absorption in the two loops.

The ^{45}Ca absorption coefficients in the two loops were not significantly different after a 1-h treatment (table 1). On the other hand the coefficient in the

proximal loop was significantly higher than in the distal loop, after a 4-h treatment (P about 0.02).

TABLE 1

^{45}Ca absorption coefficients (in %) *according to the intestinal loop and treatment length.*

Treatment length	1 h	4 h
Proximal loop	28.5 ± 4.5	58.0 ± 7.0
Distal loop	25.0 ± 7.1	40.6 ± 3.8
Number of animals	8	8

Time-course of intestinal calcium absorption.

The ^{45}Ca absorption coefficient increased both in the proximal and in the distal loop when a 4-h treatment was substituted for a 1-h treatment (table 1). This increase was more significant in the proximal loop ($0.01 < P < 0.02$) than in the distal loop (P about 0.05).

The ^{45}Ca absorption coefficient in the whole intestine was multiplied by 2.5 when a 1-h treatment was replaced by a 2-h treatment, the calculated values reaching 21 % and 55 % respectively (fig. 1). This coefficient remained stable afterwards (52 % after a 4-h treatment).

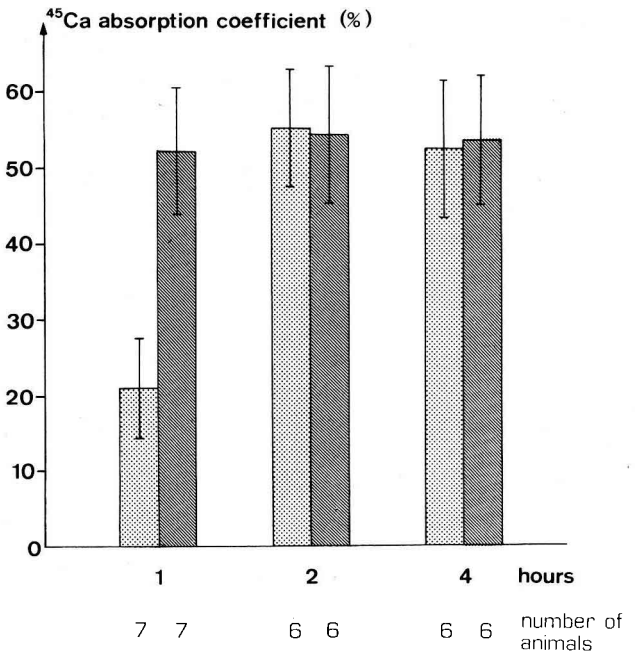

FIG. 1. — *Adult* Rana esculenta : *time course of ^{45}Ca absorption in the whole intestine in the absence* (dotted bars) *and presence* (hatched bars) *of 100 mM L-lysine in the injected solution.*

Absorbed ^{45}Ca was barely detected in the plasma and shin-bone where the radioactivity hardly exceeded the sensitivity threshold of the counter.

Effect of L-lysine on intestinal calcium absorption.

Calcium absorption in the whole intestine increased early (fig. 1) when the $CaCl_2$ solution injected into the intestinal lumen also contained L-lysine at a 100 mM concentration. Indeed ^{45}Ca absorption alreary reached its maximum value (52 %) after a 1-h treatment and was significantly higher than without the amino acid ($0.01 < P < 0.02$). Intestinal calcium absorption ceased afterwards, as demonstrated by the stability of the ^{45}Ca absorption coefficients (2 h : 54 % ; 4 h : 53 %).

Discussion and conclusion.

During its intestinal absorption, calcium crosses the microvillus membrane and cytosol and is thereafter released into the plasma at the basal pole level of the enterocyte. The intestine is the main organ concerned with calcium absorption in the frog. The absorbed calcium is deposited in the skeleton and in the « chalky » and endolymphatic sacs via plasma transport proteins. The broad outlines of the regulation of intestinal calcium absorption in frogs are known (Dacke, 1979 ; Taylor, 1985). Injected vitamin D_3 increases the calcium transport capacity of the intestine, but this response is prevented if the parathyroid glands are first removed (Robertson, 1974). Parathyroid hormone could stimulate the enzymatic conversion of 25-hydroxyvitamin D_3 into its active metabolite (1-25 dihydroxy-bitamin D_3) which seems to increase intestinal calcium absorption, as demonstrated in mammals (Pansu *et al.*, 1981).

In *Rana esculenta* as in mammals, intestinal calcium absorption is not complete after a chalky solution is injected into the lumen. Nevertheless absorption goes on longer in frogs (2 h) than in mammals (30 min in the rat : Dupuis *et al.*, 1980a). The ^{45}Ca absorption coefficient remains stable after a 2-h treatment.

In mammals the permeability of the intestinal microvillus membrane to calcium seems to be related to the degree of phosphorylation of certain membrane proteins. Various phosphorylable compounds injected into the intestinal lumen (sorbitol, lactose, lysine, creatine, ...) stimulate the membrane permeability to calcium (Fournier, 1954 ; Wasserman *et al.*, 1956 ; Dupuis *et al.*, 1978, 1980b ; Tardivel *et al.*, 1979 ; Landiharintsoa, 1981 ; Fournier and Dupuis, 1982). The stimulation could be associated with a decrease in membrane protein phosphorylation resulting from a competition effect. Conversely, the phosphates found in the intestinal lumen, wich might increase the degree of phosphorylation of membrane proteins, reduce calcium absorption. Such a correlation is not specific of the intestinal microvillus membrane, since it has also been shown in the membranes of the outer segment disks of the retinal rod (Weller *et al.*, 1975) and in those of the synaptosomes (Weller and Morgan, 1977) and erythrocytes (Weller and Laing, 1978).

In the frog (*Rana esculenta*), such a correlation has been demonstrated in the gut by injecting L-lysine into the intestinal lumen. Indeed this amino acid

decreases the degree of phosphorylation of the microvillus membrane proteins (El Maraghi-Ater *et al.,* 1986) but it increases the rate of intestinal calcium absorption, as shown by our results. In the rat it should be emphasized that this molecule does not have any effect on the rate of intestinal calcium absorption, but extends the duration of this phenomenon (Dupuis *et al.,* 1980a).

The mechanisms which associate the degree of phosphorylation of intestinal microvillus membrane proteins with the permeability of this membrane to calcium are still hypothetic. According to Dupuis *et al.* (1981), the main phosphorylable protein in the rat intestinal microvillus membrane seems to be alkaline phosphatase. In *Rana esculenta* the main phosphorylable protein seems also to be alkaline phosphatase (El Maraghi-Ater *et al.,* 1986), which might play a major role in controlling the permeability of intestinal microvillus membrane proteins to calcium.

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Résumé. *Variations de l'absorption intestinale du calcium chez* Rana esculenta. *Effet de la lysine.*

Les valeurs de l'absorption du calcium intestinal ont été recherchées chez une grenouille adulte (*Rana esculenta*), en injectant dans la lumière une solution de $CaCl_2$ contenant du ^{45}Ca. Le coefficient d'absorption du ^{45}Ca est plus élevé dans l'anse proximale que dans l'anse distale, lorsque la solution de $CaCl_2$ est maintenue pendant 4 heures dans la lumière intestinale. Ce coefficient augmente à la fois dans les anses proximale et distale, quand un traitement de 4 h est substitué à un traitement de 1 h. Le coeficient s'élève dans l'intestin entier durant les 2 premières heures de traitement (1 h : 21 %, 2 h : 55 %) puis demeure stable. En présence de L-lysine (100 mM), l'absorption intestinale du calcium s'accélère précocement, puisque le coefficient a déjà atteint sa valeur maximale (52 %) après 1 h de traitement.

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