PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 602–610 (1986)

# The Kinetics of Intestinal Calcium Absorption in the Rat: An Analytical and Model Building Study (42298)

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*Abstract.* The experimental data obtained from *in vivo* single pass perfusion of duodenal, jejunal, and ileal intestinal segments of 33- and 50-day-old rats have been used to test a series of models for calcium absorption. Each model was checked for the statistical validity and goodness-of-fit with the experimental data. The model adopted for the duodenum and jejunum had two major components, one saturable and the other nonsaturable, and a minor secretory component. This model was not applicable to ileal calcium absorption. Here the secretory component appeared to be much more important, and the absorption parameters varied in such a manner as to suggest that this intestinal segment was capable of short term autoregulation of dietary calcium absorption.


A variety of techniques have been used to investigate the mechanisms of intestinal calcium transport and some of the most sophisticated experiments have been carried out *in vitro* (1, 2). The results have demonstrated the complexity of the phenomena involved, particularly when the overall process was considered: trans/paracellular pathways, energy-dependent and independent transports, simple or facilitated diffusion. This complexity has been confirmed *in vivo* in so far as the kinetic studies have shown the existence, at least in the proximal segments of intestine, of two different types of transport, the first nonsaturable, the second saturable, which can be approximated to simple diffusion and mechanisms such as facilitated diffusion requiring carriers. The kinetic studies on the chicken (3) and on the rat (4, 5) have suggested a theoretical model for calcium transport which involved both mechanisms. The relative importance of each mechanism varies from one part of the small intestine to another. While most authors agree that simple diffusion occurs throughout the length of the small intestine, they hold divergent views as to the presence (4) or absence (6) of a saturable component in the ileum. As it is generally believed that the ileum plays an important role in the absorption of dietary calcium (7), the presence or absence of a carrier-based facilitated diffusion mechanism could have considerable significance for the regulation of calcium uptake in this intestinal segment. Studies of the effects of vitamin D on ileal calcium uptake have provided contradictory results. Pansu *et al.* (6) found no modification in absorption while others (8–10) observed increased absorption. Nevertheless, it has been clearly demonstrated (11) that the ileum is capable of adaptation to changes in dietary content.

Initially our study was undertaken to verify the presence of a saturable component in calcium transport systems of the ileum. For this purpose an experimental design close to that of Wilkinson (4) was adopted, as it allowed the measurement of both input and output fluid concentrations. The data obtained for duodenum and jejunum were analyzed using a modeling procedure which satisfied the theoretical requirements necessary for the model to be valid, nonlinear regression and statistical validation of the parameters (12). The modeling procedure, and the manner in which the experimental data can be interpreted by it, confirm the existence, at least for the proximal small intestine, of the two types of transport which have been previously proposed. However, the data obtained for the distal ileum indicate that conditions in this segment are different. Our interpretation of calcium movements in the ileum leads us to suggest that a rather unusual type of autoregulation occurs in this segment.

**Material and Methods.** *I. Experimental study. (i) Surgical procedure.* Male Wistar CF rats, ages 33 and 50 days, were maintained on a standard laboratory diet (UAR Villemois-



0037-9727/86 $1.50

son-sur-Orge, France) containing 6 g of calcium and 1500 UI of vitamin D/kg and given water *ad libitum*. They were housed in a temperature-controlled room (22°C) with alternate 12-hr light/dark cycles. Experiments were performed at the end of the morning on unfasted rats.

The animals were anesthetized with Thiopental (6 mg/100 g body wt). A central longitudinal incision was made in the abdominal wall, and the biliary duct and pylorus were ligated. Seven- to ten-centimeter-long segments of duodenum (from 1 cm distal to the pylorus to the angle of Treitz), jejunum (from the angle of Treitz to 7–10 cm distal), or terminal ileum were cannulated with Silastic catheters (i.d. 1.5 mm, o.d. 3.5 mm) which were introduced through a small opening on the antimesenteric side of the intestine and fixed in place by a ligature. The cannulated intestinal loop (one per rat) was then replaced inside the abdominal cavity, which was closed with sutures, and the rats were placed with the ventral surface on a heated plate to maintain their body temperature at about 37°C throughout the study. The input catheters were connected to a multichannel pump; the output catheters, cut to a minimal length to reduce dead volume, were passed through a hole in the heated plate, and the effluents from each rat were collected.

*(ii) Solutions.* Increasing quantities of 40 $CaCl_2$ were added to a pH 7.4 buffer (25 m*M* Tris–HCl, 40 m*M* NaCl) to obtain calcium concentrations of 0.3125, 0.625, 1.25, 2.5, 5, 10, 20 m*M*, 45 $CaCl_2$ (10 $\mu$Ci/m*M* of 40 Ca) was added as tracer. Final molarity was adjusted to 300 mosmol with choline chloride, and phenol red was added to allow estimation of water flux across the intestinal mucosa.

*(iii) Perfusion.* The luminal contents of the rats' intestinal segments were flushed out by rapid perfusion with the buffer solution. Each of the different calcium solutions, starting with the least concentrated, was then perfused at 37°C. Each perfusion comprised an initial 4 min at high flow rate (2 ml $\cdot$ min$^{-1}$), to eliminate the preceding solution followed by 5 min at low flow rate (0.2 ml $\cdot$ min$^{-1}$), the time required to obtain a constant value of effluent concentration, as determined in preliminary tests. The effluent was collected during the ensuing 5 min. At the end of the experiment the perfused intestinal segments were stripped from the mesentery and cut at the inlet and outlet catheters. They were quickly blotted on filter paper and weighed immediately. The 40 Ca concentrations in input ($C_i$) and output ($C_o$) fluids were determined by atomic absorption spectrometry; 45 Ca was measured by liquid scintillation. Water fluxes, estimated from changes in phenol red concentration, were negligible under our experimental conditions.

*II. Data analysis and modeling.* The experimental results were compared with a series of models of increasing complexity.

*(i) Models.* We assumed the involvement of one or more of the following transport mechanisms.

(a) Simple diffusion. The flux $J_d$ is proportional to lumen Ca concentration ($Ca_L$): $J_d = D[Ca_L]$ where $D$ is expressed in ml $\cdot$ hr$^{-1}$ $\cdot$ g$^{-1}$ (wet tissue) and $J_d$ in $\mu M \cdot hr^{-1} \cdot g^{-1}$.

(b) Saturable transports, represented by the general expression, deduced from the Adair equation (13)

$$J = \sum_{m=1}^{M} J_{max} \frac{\sum_{n=1}^{N} n \times [Ca_L]^n \times \prod_{j=1}^{n} K_j}{1 + \sum_{n=1}^{N} [Ca_L]^n \times \prod_{j=1}^{n} K_j}$$

where we assume $M$ carriers with $N$ independent binding sites. $J_{max}$ is maximal velocity ($\mu M \cdot hr^{-1} \cdot g^{-1}$), $K_j$ is constant (m$M^{-1}$); $J$ is expressed in $\mu M \cdot hr^{-1} \cdot g^{-1}$. Two specific forms of this expression have been used in this study: Case 1. One or two saturable carriers with one binding site each (i.e., $M = 1$ or $M = 2$ and $N = 1$)

$$J = \sum_{m=1}^{M} J_{max} \frac{[Ca_L]}{K_m + [Ca_L]}$$

where $K_m = 1/K_1$ is the Michaelis constant (m$M$). Case 2. One saturable carrier with two independent binding sites ($M = 1$ and $N = 2$):

$$J = J_{max} \frac{[Ca_L] + 2K_2[Ca_L]^2}{K + [Ca_L] + K_2[Ca_L]^2}$$

$$\text{with} \quad K = \frac{1}{K_1}.$$

(c) Secretion of calcium into the lumen, $J_s$, was assumed to be proportional to plasma cal-

cium concentration which has been shown to be constant in preliminary experiments. $J_s$ is expressed in $\mu M \cdot hr^{-1} \cdot g^{-1}$.

Assuming that $[Ca_L] = C_i$, the 40 Ca concentration in the effluent, $C_o$, by the general formula

$$C_o = \frac{1}{\delta}[J_s + (\delta - D)C_i - J]$$

where $\delta$ is the perfusion flow rate ($ml \cdot hr^{-1} \cdot g^{-1}$). An identical expression was used for 45 Ca concentration, with the assumption of $J_s = 0$ (negligible 45 Ca plasma concentration). This is the most complex general expression but the optimization procedure consisted of verifying the need for each parameter; we increased the complexity of the tested model until the corresponding criterion decreased and did not significantly differ from the theoretical criterion (see Data Analysis).

*(ii) Data analysis.* For a given model, the fitting of the experimental data was carried out by nonlinear regression. This procedure was based on the iterative use of two optimization algorithms, those of Vignes (14), and those of Rosenbrock (15). The criterion was the sum of the two partial criteria for 40 Ca and 45 Ca concentration, respectively. Each partial criterion was

$$WMSS = WESS + WRSS$$

where WMSS = weighted model sum of squares; WESS = weighted experimental sum of squares; WRSS = weighted residual sum of squares.

The weight is $1/(\sigma_i^2 + \sigma_o^2)$, where $\sigma_i$ and $\sigma_o$ are the input and output concentration variances, respectively. WESS is a constant value, equal to the number of experimental measurement; so only WRSS may be minimized, according to the expression

$$WRSS = \sum_1^S \frac{N^2}{N-1} \times \frac{(\hat{m} - \bar{m})^2}{\sigma_i^2 + \sigma_o^2}$$

where $\hat{m}$ = model response, $\bar{m}$ = experimental mean, $N$ = number of measurements for each solution, and $S$ = number of solutions; which, for a normal distribution, is the maximum of likelihood. The distance $(\hat{m} - \bar{m})$ is estimated geometrically according to Lybanon's method (16).

*(iii) Model selection.* The choice of a model was based on the consideration of the two following indices: (a) ability to fit the available data, and (b) accuracy associated with the parameter values.

(a) The goodness-of-fit was tested by computing the $F$ value such as

$$F = \frac{WRSS/(S-P)}{WESS/(\sum N - S)}$$

where $P$ is the number of model parameters. The model was judged to be adequate if $F < F(S - P, \sum N - S, \alpha < 0.05)$.

(b) The accuracy of parameters was determined by the classical variance–covariance matrix (17); this accuracy must be such that the parameter value differs significantly from zero, i.e., relative accuracy $\times$ 100 < 100).

$$\frac{t \times SEM}{p} \times 100 < 100$$

where $t$ = Student $t$ value for 0.05, with $df = (S - P)$, $p$ = parameter value, and SEM = standard error of $p$.

*(iv) Comparison of values.* The comparison of the same parameter values, obtained from different experimental data, was carried out using a $t$ test.

**Results.** *I. Duodenal calcium absorption: 50-day-old rats.* The 40 Ca and 45 Ca concentrations in input and output fluids are reported in Table I. Results from the modeling carried out with these experimental data (Table II) show that only models V and VII simultaneously satisfy the chosen indices. Both models combine two distinct transport mechanisms and a constant secretion of calcium into the lumen.

In model V the saturable transport is associated with simple diffusion (see Fig. 1 for global behavior and respective contributions to absorption rate of saturable and nonsaturable components, such as predicted by model V for the optimized parameter values), while in model VII it is associated with a second saturable transport having a high $K_m$ value. Nevertheless it should be noted that the uncertainty of this $K_m$ is high, close to maximum acceptable value (93 for a max of 100).

*II. Duodenal and jejunal calcium absorption: 33-day-old rats.* Models V and VII were

TABLE I. $^{40}Ca$ AND $^{45}Ca$ CONCENTRATIONS IN THE FLUIDS ENTERING ($C_i$) AND LEAVING ($C_o$) THE DUODENAL LOOPS OF 50-DAY-OLD RATS (EXPERIMENT 1)

| $^{40}Ca$ (m$M$) | | $^{45}Ca$ ($10^4$ cpm/ml) | |
|---|---|---|---|
| $C_i$[a] | $C_o$[b] | $C_i$ | $C_o$ |
| 0.332 ± 0.17 | 0.255 ± 0.024 | 2.04 ± 0.029 | 1.44 ± 0.17 |
| 1.29 ± 0.02 | 1.07 ± 0.06 | 8.47 ± 0.17 | 6.75 ± 0.37 |
| 2.59 ± 0.06 | 2.24 ± 0.09 | 16.69 ± 0.42 | 14.33 ± 0.66 |
| 5.15 ± 0.12 | 4.67 ± 0.15 | 33.78 ± 0.25 | 30.16 ± 0.87 |
| 10.37 ± 0.26 | 9.59 ± 0.26 | 67.85 ± 0.49 | 62.42 ± 0.90 |
| 20.45 ± 0.55 | 19.33 ± 0.47 | 135.46 ± 1.26 | 128.03 ± 1.18 |

[a] $C_i$ results are mean values ± SD for three determinations of one sample.
[b] $C_o$ results are expressed as mean values ± SD for 5 rats.

TABLE II. RESULTS OF THE MODELING PROCEDURE

| Model | Number and specificity of included mechanisms: Simple diffusion | Saturable transport: Michaelis | Saturable transport: Adair | Secretion | Parameter estimate: Values | Parameter estimate: Relative accuracy × 100 | Criterion: Partial $^{40}Ca$ | Criterion: Partial $^{45}Ca$ | Criterion: Total |
|---|---|---|---|---|---|---|---|---|---|
| I | 1 | 0 | 0 | 0 | $D$ = 2.13 | 2.99 | 219.05* | 480.56* | 699.61* |
| II | 1 | 0 | 0 | 1 | $D$ = 2.13 | 3.12 | 219.12* | 480.49* | 699.61* |
| | | | | | $J_s = 5.19 \times 10^{-8}$ | $>10^2$ | | | |
| III | 0 | 1 | 0 | 0 | $J_{max}$ = 31.17 | 7.36 | 20.76* | 40.89* | 61.65* |
| | | | | | $K_m$ = 5.39 | 12.04 | | | |
| IV | 1 | 1 | 0 | 0 | $D$ = 0.95 | 17.24 | 9.60 | 13.36* | 22.96* |
| | | | | | $J_{max}$ = 11.95 | 21.13 | | | |
| | | | | | $K_m$ = 1.76 | 28.48 | | | |
| V | 1 | 1 | 0 | 1 | $D$ = 1.00 | 14.95 | 4.14 | 5.73 | 9.87 |
| | | | | | $J_{max}$ = 10.49 | 17.93 | | | |
| | | | | | $K_m$ = 1.29 | 28.64 | | | |
| | | | | | $J_s$ = 0.61 | 49.20 | | | |
| VI | 0 | 2 | 0 | 0 | $J_{max}$ = 52.95 | 48.97 | 8.97* | 9.86* | 18.83 |
| | | | | | $K_{m_1}$ = 26.16 | $>10^2$ | | | |
| | | | | | $J_{max}$ = 5.98 | 75.54 | | | |
| | | | | | $K_{m_2}$ = 0.94 | 78.08 | | | |
| VII | 0 | 2 | 0 | 1 | $J_{max_1}$ = 52.69 | 47.16 | 2.06 | 2.16 | 4.22 |
| | | | | | $K_{m_1}$ = 25.70 | 93.61 | | | |
| | | | | | $J_{max_2}$ = 5.89 | 58.30 | | | |
| | | | | | $K_{m_2}$ = 0.71 | 68.73 | | | |
| | | | | | $J_s$ = 0.64 | 48.11 | | | |
| VIII | 1 | 0 | 1 | 1 | $D$ = 0.82 | 45.74 | 3.19 | 4.06 | 7.25 |
| | | | | | $J_{max}$ = 8.28 | 54.75 | | | |
| | | | | | $K$ = 0.95 | 44.56 | | | |
| | | | | | $K_2$ = 32.90 | $>10^2$ | | | |
| | | | | | $J_s$ = 0.63 | 49.30 | | | |

*Note.* $D$ = diffusion constant (ml · hr$^{-1}$ · g$^{-1}$). $J_s$ = unidirectional flux of Ca from tissue to lumen ($\mu M$ · hr$^{-1}$ · g$^{-1}$). $J_{max}$, $J_{max_1}$, $J_{max_2}$ = maximal velocities ($\mu M$ · hr$^{-1}$ · g$^{-1}$). $K_m$, $K_{m_1}$, $K_{m_2}$ = Michaelis constants (m$M$). $K = 1/K_1$ = constant (m$M$). $K_2$ = constant (m$M^{-1}$).
* $F$ superior to the maximal theoretical value ($\alpha < 0.05$).

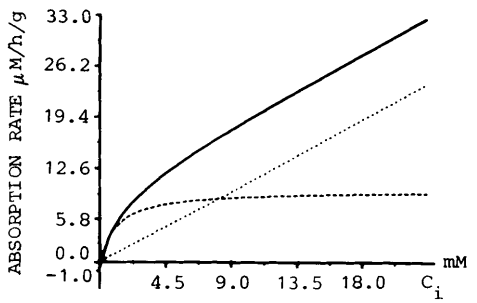


FIG. 1. Simulated changes in the calcium absorption rate as a function of input fluid calcium concentration ($C_i$) with respective components of diffusion (· · ·) and saturable transport (---). This behavior was obtained from Model V for the values of parameter given in Table II (50-day-old rats).

tested with experimental data on calcium transport in duodenal and jejunal loops of 33-day-old rats. The accuracy of the parameter values confirmed the validity of model V for these segments of intestine and led us to reject model VII, whose parameters did not differ significantly from 0. Among the parameter values, the $J_{max}$ value differed significantly between jejunum and duodenum (3.22 versus 6.16 $\mu M \cdot hr^{-1} \cdot g^{-1}$), as did $K_m$ (0.67 versus 1.41 m$M$); while $D$ and $J_s$ were not significantly modified. Similarly, the values of $D$ and $J_s$, estimated for the duodenum of 33-day-old rats, did not vary significantly from those of 50-day-old rats. In this comparison, only $J_{max}$ decreases (6.16 $\mu M \cdot hr^{-1} \cdot g^{-1}$ versus 10.49), since $K_m$, in this case, did not change significantly.

*III. Ileal calcium absorption: 33-day-old rats.* The effect of increasing the cold calcium concentration of the perfusing fluid ($C_i$) on calcium absorption, as measured by the difference between input ($C_i$) and output ($C_o$) fluid calcium concentrations, is shown in Fig. 2. The concentration differences for both calcium (40 Ca) and tracer (45 Ca) are shown for the duodenum and ileum. The steady increase in labeled and unlabeled calcium absorption with rising perfusing concentrations in the duodenum is as predicted by model V. The ileal response, however, is biphasic, with an initial absorption of both labeled and unlabeled calcium, up to a perfusing concentration of 2.5 m$M$. Beyond this concentration, the two curves diverge, the tracer curve becoming not significantly different from zero value, while the cold curve takes negative values.

*IV. Test of experimental design.* The validity of the experimental design was checked by

TABLE III. PARAMETER ESTIMATES FROM MODEL V FOR DUODENAL AND JEJUNAL LOOPS OF 33-DAY-OLD RATS

| | Parameter estimates | |
|---|---|---|
| Model V | Values | Relative accuracy × 100 |
| Duodenal loops (33-day-old rats) | $J_{max}$ = 6.16** | 27.16 |
| | $K_m$ = 1.41* | 48.94 |
| | $D$ = 0.91 | 16.20 |
| | $J_s$ = 0.51 | 71.45 |
| Jejunal loops (33-day-old rats) | $J_{max}$ = 3.22** | 20.84 |
| | $K_m$ = 0.67* | 39.04 |
| | $D$ = 1.07 | 11.57 |
| | $J_s$ = 0.37 | 40.66 |

*Note.* $J_{max}$ = maximal velocities ($\mu M \cdot hr^{-1} \cdot g^{-1}$), $K_m$ = Michaelis constants (m$M$), $D$ = diffusion constant ($ml \cdot hr^{-1} \cdot g^{-1}$), $J_s$ = unidirectional flux of Ca from tissue to lumen ($\mu M \cdot hr^{-1} \cdot g^{-1}$).

* $0.02 < \alpha < 0.05$.

** $\alpha < 0.001$.

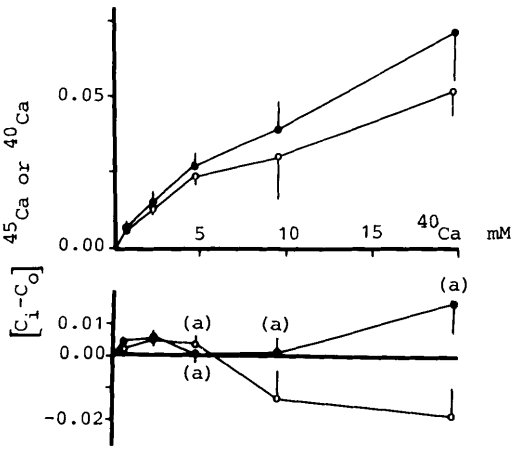


FIG. 2. Effect of $^{40}Ca$ concentrations in input fluid ($C_i$) on calcium kinetics of duodenal (upper panel) and ileal (lower panel) loops in 33-day-old rats. The results are expressed as the difference between $^{45}Ca$ (●) or $^{40}Ca$ (○) concentration in input ($C_i$) and output ($C_o$) fluids. The concentrations were normalized to their maximal values. Mean values (four rats per group and three determinations for each sample) ± SD. (a) Not significantly different from zero.

sequential perfusion with the 2.5 and 20 m*M* calcium concentrations. Duodenal (three rats) and ileal (four rats) loops were perfused with 2.5 m*M* solution for 10 min and with 20 m*M* solution for 10 min. The perfusions were repeated in the same order. The absorption capacity, taken as the difference $\Delta C = C_o - C_i$, was measured for each perfusing solution and the values obtained for the first, $\Delta C_1$ and the second, $\Delta C_2$, passages were compared (Table IV). The values of $\Delta C_1$ and $\Delta C_2$ obtained for the low calcium solutions in the duodenum did not differ significantly. Similarly, the duodenum values of $\Delta C_1$ and $\Delta C_2$ for the high calcium solution indicated that the absorption capacity of the duodenum did not change during the test. The values of all the $\Delta C_1$ and $\Delta C_2$ were significantly different from 0. However, the values for $\Delta C_1$ and $\Delta C_2$ obtained after perfusion of the ileum were significantly different. $\Delta C_2$ was significantly lower than $\Delta C_1$ and was not different from 0.

**Discussion.** We have used perfused *in vivo* intestinal loops and model building techniques to establish and test a quantitative model for the intestinal absorption of calcium. The design of the theoretical model involved the application of (a) nonlinear regression analysis, whose advantages have been emphasized by Gardner and Atkin (12); (b) choice of a criterion: the original data, $C_i$ and $C_o$, weighted for the uncertainties of these values, were used rather than transformed data (e.g., estimation of absorption rates). It was then possible to check, statistically, the goodness of fit; and (c) determination of parameter value accuracy, allowing the rejection of models whose complexity was not justified.

The resulting model for the duodenum and jejunum included a saturable transport component, a nonsaturable transport component, and the constant secretion of calcium into the intestinal lumen. It corresponds to the classical model proposed by Wasserman in chick intestine (3) and adopted by some authors for the rat small intestine (4, 5). Saturable transport is explained by the involvement of biological material, whose quantity is rate limiting. Most authors agree that, for calcium absorption, saturable transport could be a facilitated diffusion process operating down a concentration gradient through either a "carrier," or more probably, electrically conducting pores. Thus, the $J_{max}$ and $K_m$ parameters take on a precise meaning: $J_{max}$ is related to total quantity of available "carrier," and $K_m$ is a specific characteristic of that carrier.

Therefore, variations in the saturable transport capacity may be due to variations of $J_{max}$, with or without modifications of $K_m$. However, some experimental results make a case for the direct or indirect implication of energy-dependent processes (18). Nonsaturable transport may be interpreted as simple diffusion. As the transport of inorganic ions through cell membranes without a facilitated diffusion remained in doubt, it has been suggested that simple diffusion acts through the tight junction in leaky epithelia (19). The discrimination between such a process and a sat-

TABLE IV. VARIATIONS IN DUODENAL AND ILEAL ABSORPTION CAPACITIES INDUCED BY A FIRST PERFUSION OF 2.5 AND 20 m*M* CALCIUM SOLUTIONS

| | $\Delta C = C_o - C_i$ (m*M*) | | | |
|---|---|---|---|---|
| | Duodenum (3)[c] | | Ileum (4)[c] | |
| $C_i$ (m*M*) | $\Delta C_1$ | $\Delta C_2$ | $\Delta C_1$ | $\Delta C_2$ |
| 2.5 | −0.81 ± 0.06 | −0.75 ± 0.08 | −0.48 ± 0.07 | −0.14 ± 0.05[a,b] |
| 20 | −3.27 ± 0.39 | −2.88 ± 0.28 | −2.56 ± 0.70 | +0.94 ± 0.65[a,b] |

*Note.* Absorption capacity was taken as the difference between the 40 calcium concentrations input ($C_i$) and output ($C_o$) fluids. The first passage consisted of the successive 10-min perfusion of 2.5 and 20 m*M* calcium solutions. The second passage duplicated this experimental procedure.

[a] Significant difference versus first passage, $P < 0.01$.

[b] $C_i$ not significantly different from $C_o$.

[c] Mean values ± SD, number of rats indicated in parentheses.

urable transport with a high $K_m$ is known to be sometimes difficult (12).

A calcium secretion component has been included in the model. While the values for this component were very small, its inclusion was statistically justified.

The results obtained from duodenum transport data give rise to the following points: (i) Interestingly, the values obtained in this study for $J_{max}$ and $K_m$, by measuring calcium concentrations in perfusates, are in agreement with those obtained by Papworth and Patrick (18) from measurements of calcium uptake by duodenal slices, *in vitro* ($J_{max}$ = 11.41 $\pm$ 0.49 $\mu M \cdot hr^{-1} \cdot g^{-1}$ and $K_m = 1.15 \pm 0.07$ m$M$). (ii) In the same way the orders of magnitude of the identified parameter values for duodenum were consistent with the results of Wilkinson, obtained *in vivo;* $J_{max}$, $K_m$, $D$, and $J_o$, when expressed in our units, were about 5.0, 1.0, 0.6, and 1, respectively (4). (iii) The only parameter for duodenal calcium absorption which varies between 33-day-old and 50-day-old rats is $J_{max}$. This result is in agreement with those of authors studying saturable transport (4, 18). (iv) The increase of $J_{max}$ between 33 and 50 days may appear surprising as it is generally believed that calcium absorption decreases with age. However, this higher value may be interpreted as the increase of active (20) or saturable (21) transport during the postweaning weeks. While this result does not fully agree with that of Pansu *et al.* (21), a quantitative comparison of our parameter values with their results is not possible, because their experimental design led them to express their parameters as quantities of calcium and not as concentrations of calcium.

The validity of our model for jejunum is in agreement with the results of various authors (6, 22) providing evidence for calcium transport in this intestinal segment. The lower values of $J_{max}$ in jejunum compared to duodenum confirm previous observations (4, 6, 18) and could be related to the lower calcium uptake capacity of isolated jejunal cells compared to duodenal cells (23). The barely significant variation in $K_m$ between jejunal and duodenal tissues could suggest a discrepancy of the "carrier" characteristics.

Thus, the adopted model satisfies both the qualitative and quantitative criteria for calcium transport in the duodenum and jejunum. The data obtained from the ileum perfusion cannot be interpreted with the model because the parameters contributing to the global process apparently changed during the experiment. A different experimental design, for instance, using different rats for each solution (4), may have provided experimental data which allowed the application of the model to the ileum, and the evaluation of its transport parameters. Indeed, the results of the complementary experiments fully support this point. During the first passage of 20 m$M$ fluid we observed that calcium transport mechanisms are present, while during the second passage of the same fluid, they were absent.

This observation led us to postulate that changes in transport capacity are only indirectly dependent on the intraluminal calcium, which exerts its effects through modification of the properties of intestinal tissue. Such modifications could include the following: (i) A variation of the calcium secretion into the lumen, whose importance may depend on the intraluminal concentration. Thus, the increase of 40 Ca concentration for the second part of the experiment on ileal loops may be interpreted as an increase of secretion in agreement with the observations of Dumont *et al.* on rat ileum (24) and Wasserman on chicken duodenum (25). Nevertheless, this increase of calcium secretion into the lumen does not explain the change of 45 Ca concentrations, because in this case, it would be necessary to imagine a nonnegligible return of 45 Ca into the lumen, i.e., an absence of dilution or utilization beyond the epithelium itself. (ii) If, as is generally agreed, saturable transport plays a minor role in ileal calcium absorption (6), we are left with the possibility that the simple diffusion process itself is regulated. The structure of the tight junctions, whose possible role has been previously emphasized, is closely linked to the extracellular and/or cytosolic calcium concentration(s) (26, 27). It seems reasonable to assume that the tight junctions are more permeable to ion fluxes at low concentrations. For our results, taking into account the time required for junctional integrity as measured *in vitro* (26), this may be an effect of cytosolic Ca concentration, rather than a direct effect of intraluminal concentrations, in agreement

with the conclusions of Palant *et al.* (27) on calcium function in tight junctional integrity of leaky epithelia.

Such a role of cytosolic calcium could explain the difference between the behaviors of the ileum and the duodenum–jejunum. These later segments are generally regarded as possessing not only more developed intracellular calcium "buffering" capacities (28, 29), but also higher extrusion capacities from epithelium to serosa (30).

Our results confirm the higher calcium absorptive capacity of duodenal tissue as compared to the ileum. The results obtained in the ileum are suggestive of a type of regulation of calcium flux from the lumen to the plasma; this regulation probably does not involve hormonal regulation but is influenced, either directly or indirectly, by intraluminal calcium concentration. Thus, when concentration rises the ileal absorption capacity decreases. This type of regulation is consistent with the observations of Petith and Schedl (11). Their studies on rats fed various calcium diets led them to conclude that calcium absorption rather than calcium secretion was regulated. This adaptation could be obtained as early as the first high calcium alimentary bolus and before the vitamin D-dependent adaptation of the proximal small intestine (decrease of the saturable component (6)). Regulation of this type could be most efficient as the ileum plays a relatively important role in dietary calcium absorption; its low absorptive capacity being more than offset by its length (7, 11).

We are indebted to Dr. C. O. Parkes for help and comments in the preparation of the manuscript, to V. Myrtil for his expert technical assistance, and to F. Sarrazin for the secretarial assistance.

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Received February 22, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted December 31, 1985.