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# THE ACCUMULATION OF CALCIUM IN LAYING FOWL INTESTINE *IN VITRO**

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SUMMARY

Laying hen intestinal tissue was incubated in media containing $^{45}Ca^{2+}$, and the accumulation of this isotope was assessed in either the whole tissue or the mucosa. The variable osmotic agent in the medium was mannitol, and the inclusion of any substance was at its expense. The following observations were made:

1. The specific activity ($^{45}Ca^{2+}/^{40}Ca^{2+}$) of the accumulated $Ca^{2+}$ was found to be constant.

2. The intestinal accumulation of $^{45}Ca^{2+}$ was linearly related to the activity of $Ca^{2+}$ in the incubation medium.

3. Chelating agents reduced the accumulation to a lesser extent than expected on the basis of $Ca^{2+}$ activity in the medium, suggesting some accumulation of complexed Ca.

4. More $^{45}Ca^{2+}$ was accumulated from the $Na^+$-poor as compared with $Na^+$-rich medium.

5. The accumulation was higher with fructose than with glucose in the medium. Neither ouabain nor phlorizin inhibited the accumulation of $^{45}Ca^{2+}$ from an $Na^+$-poor medium.

6. Incubation of intestinal tissue in an $Na^+$-poor medium resulted in an $O_2$ consumption lower than with $Na^+$-rich medium. This effect was reversible after 30 min of incubation. Similarly, the effect of $Na^+$ on the accumulation of $^{45}Ca^{2+}$ was reversible after the same time period.

7. The accumulation was linear with respect to the $Ca^{2+}$ activity of the medium regardless of the $Na^+$ concentration.

The findings are consistent with the concept of diffusion as the mechanism for the entry of $Ca^{2+}$ into the mucosa, although they do not entirely rule out some participation of other types of transport.

## INTRODUCTION

The major mechanisms which have been considered for $Ca^{2+}$ transport were active transport[1–6] and simple or facilitated diffusion[5–8].

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SCHACHTER *et al.*[1–3] using both everted gut sacs and gut slices from rats suggested an active mechanism for $Ca^{2+}$ transport. However, such active transport could be demonstrated only with $Ca^{2+}$ concentrations lower than those found in the intestinal lumen or blood plasma[9,10] and found only in the duodenum. On the other hand, HELBOCK *et al.*[8] concluded that $Ca^{2+}$ was actively transported *in vitro* by the rat intestine only in the presence of phosphate. DUMONT *et al.*[5] suggested the existence in rat ileum *in vivo*, of a passive $Ca^{2+}$ transport at low concentrations and a carrier-mediated one at high concentrations. WASSERMAN AND KALLFELZ[6], using USSING's[11] model for the chick intestine, suggested that the transport was active at low $Ca^{2+}$ concentrations but consisted of "combinations of exchange diffusion and simple diffusion" at high $Ca^{2+}$ concentrations.

DUMONT *et al.*[5] showed that $Ca^{2+}$ in concentrations above 1.0 mM inhibited $Na^+$ transport by the rat ileum *in vivo*. Conversely, SCHACHTER *et al.*[1–3] have demonstrated both a competitive inhibition of $^{45}Ca^{2+}$ transport and an accumulation by $K^+$, $Mg^{2+}$ and $Sr^{2+}$ in the rat intestine *in vitro*. HARRISON AND HARRISON[12] and HURWITZ *et al.*[13] found a greater transmural transport of $^{45}Ca^{2+}$ *in vitro* in a medium deficient in $Na^+$ as compared with $Na^+$-rich medium for both rat and chick.

The mechanism of this inhibition could be either competition for a common carrier, as suggested by RAHILL AND WALSER[14] and PETERS AND WALSER[15], a competition between $Ca^{2+}$ and $Na^+$ at the cell membrane[5,16] or an effect of $Na^+$ on intermediary metabolism[17] altering the cell permeability to $Ca^{2+}$ (ref. 18).

Most reports on intestinal $Ca^{2+}$ transport dealt with its transmural movement. Except for some consideration by SCHACHTER *et al.*[7] and by WASSERMAN[19], $Ca^{2+}$ transport into the intestinal mucosa has not been fully studied. The first study from this laboratory[20] with laying hen intestine suggested that $Ca^{2+}$ transport into the mucosa was only slightly dependent upon an intermediary metabolism. These results were similar to those obtained *in vitro* by HARRISON AND HARRISON[12] and by WASSERMAN[19].

The present study deals mainly with the mechanism of $Ca^{2+}$ accumulation by the mucosal tissue and with the inhibition of $Ca^{2+}$ transport by $Na^+$ in the laying hen intestine.

## METHODS

### *Incubation technique*

Laying hen intestinal slices or perfused loops were prepared and incubated 20 min under $O_2$–$CO_2$ (95:5, v/v) at 39° in an isotonic buffer as described previously[20]. The buffers contained in mM: 1.0 $NaH_2PO_4$, 3.0 KCl, 0.4 $MgSO_4 \cdot 7H_2O$, 20.0 fructose, 0–130.0 NaCl, 0.5–10.0 $CaCl_2$, $NaHCO_3$ to pH 6.8, mannitol to an osmolality of 154.0 NaCl and 40–50 $\mu$C $^{45}Ca^{2+}$ per mmole $^{40}Ca^{2+}$. At the end of the incubation period, the slices were removed, washed, weighed, ashed and analyzed for $^{45}Ca^{2+}$ in a Tri-Carb liquid scintillation counter.

### *Determination of tissue $^{40}Ca^{2+}$*

Approx. 1 g of tissue was treated in a 10-ml volumetric flask with 4 ml of 20% trichloroacetic acid. The resulting precipitate was broken down by a glass rod, and the sample was brought to volume with distilled water. After 20–24 h, the contents

of the flask were centrifuged 10 min at 2000 rev./min, and the supernatant was analyzed for $^{40}Ca^{2+}$ by a direct titration with EDTA (sodium salt) under ultraviolet light in the presence of fructose and with Calcein as an indicator. $Ca^{2+}$ activity was measured with an Orion calcium electrode and Keithley electrometer model 610-B. A saturated KCl agar bridge–saturated KCl solution–calomel electrode served as a reference[10].

## RESULTS

### *$^{40}Ca^{2+}$ and $^{45}Ca^{2+}$ accumulation by the intestinal mucosa*

The use of $^{45}Ca^{2+}$ as a measure of the accumulation of $^{40}Ca^{2+}$ assumes a linear relationship between $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$ accumulation. In order to test this assumption, jejunal slices were incubated in media containing 0.5, 2.0, 4.0 or 10.0 mM $CaCl_2$. At the end of the incubation period, the mucosal tissue was scraped off the underlying tissue and was analyzed for $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$. A sample of mucosa was taken before incubation in order to determine the initial $^{40}Ca^{2+}$ concentration.

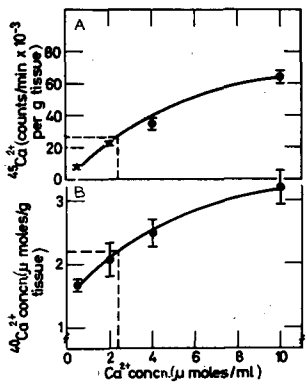



Fig. 1. Tissue $^{40}Ca^{2+}$ (B) and $^{45}Ca^{2+}$ (A) concentration as influenced by $Ca^{2+}$ concentration from medium. The specific activity of the media was $4.1 \cdot 10^4$ counts/min per μmole $^{40}Ca^{2+}$. Each value is an average ± S.E. of 10 birds. Initial tissue $Ca^{2+}$ concentration was 2.21 μmoles/g. The $Ca^{2+}$ in the medium which maintained this concentration during incubation was graphically estimated at 2.4 mmoles/l (–––––, B). The $^{45}Ca^{2+}$ concentration in the tissue at that medium concentration was $27 \cdot 10^3$ counts/min per g (–––––, A).

Tissue $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$ concentrations were plotted against the $^{40}Ca^{2+}$ concentration of the media (Fig. 1). In order to make the slopes of both plots comparable, the scale of the ordinate in the $^{45}Ca^{2+}$ plot over the ordinate scale in the $^{40}Ca^{2+}$ plot was set at the specific activity of the medium ($4.1 \cdot 10^4$ counts/min per μmole). It is apparent that the curves of both tissue $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$ are parallel. Furthermore, the specific activity of the accumulated $Ca^{2+}$ (Table I) was constant in three of the four media and was close to the specific activity of the media. On the other hand, the specific activity of the tissue did not reach that of the medium during the 20-min incubation period.

Since the changes in tissue $^{45}Ca^{2+}$ reflected those of $^{40}Ca^{2+}$, it may be concluded that under such conditions, the accumulation of $^{45}Ca^{2+}$ can be used as a measure of $^{40}Ca^{2+}$ accumulation.

TABLE I

SPECIFIC ACTIVITY OF $Ca^{2+}$ IN INTESTINAL MUCOSA

Each value is the average of 10 slices from 10 birds ± S.E. The specific activity of the media was $4.1 \cdot 10^4$ counts $^{45}Ca^{2+}$ per min per μmole $^{40}Ca^{2+}$.

| $Ca^{2+}$ concn. (mM) | Specific activity (counts/min × $10^{-3}$ per μmole $Ca^{2+}$) | |
|---|---|---|
| | $^{45}Ca^{2+}/^{40}Ca^{2+}$ * | $\Delta^{45}Ca^{2+}/\Delta^{40}Ca^{2+}$ ** |
| 0.5 | 4.7 ± 0.50 | 36.4 |
| 2.0 | 11.6 ± 1.00 | 43.7 |
| 4.0 | 14.6 ± 1.60 | 25.0 |
| 10.0 | 20.7 ± 1.60 | 37.1 |

* Total tissue $^{45}Ca^{2+}$/total tissue $^{40}Ca^{2+}$.

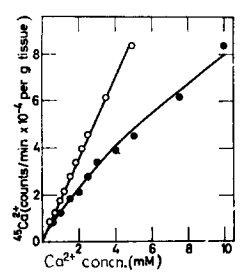
** $\Delta^{40}Ca^{2+} = {}^{40}Ca_t{}^{2+} - {}^{40}Ca_o{}^{2+}$; $\Delta^{45}Ca^{2+} = {}^{45}Ca_t{}^{2+} - {}^{45}Ca_b{}^{2+}$. $^{40}Ca_t{}^{2+}$ and $^{45}Ca_t{}^{2+}$ are, respectively, the $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$ concentrations in the tissue after 20 min of incubation. $^{40}Ca_o{}^{2+}$ is the initial $^{40}Ca^{2+}$ concentration in the tissue. $^{45}Ca_b{}^{2+}$ is the $^{45}Ca^{2+}$ concentration in the tissue maintained at the initial $^{40}Ca^{2+}$ concentration during incubation; estimated graphically from Fig. 1.

With the lowest concentration of $Ca^{2+}$ in the medium, the tissue lost a considerable portion of its initial 2.21 μmoles/g $Ca^{2+}$, whereas in the two media with the highest concentrations the tissue accumulated $Ca^{2+}$. The $Ca^{2+}$ concentration in the medium which maintained $Ca^{2+}$ in the tissue at its initial value (balance concentration) was estimated from the $^{40}Ca^{2+}$ plot to be 2.4 μmoles/ml. Both tissue and medium concentrations are remarkably similar.

*The dependence of $^{45}Ca^{2+}$ accumulation on $Ca^{2+}$ concentration of the medium*

Several experiments were conducted in order to determine the relationship of the $^{45}Ca^{2+}$ accumulation by intestinal slices to the concentration or activity of $Ca^{2+}$ in the medium. Jejunal slices were incubated in media containing 0.5–10.0 mM $Ca^{2+}$.

The $^{45}Ca^{2+}$ accumulation as a function of $Ca^{2+}$ concentration in the media appeared to be curvilinear, whereas a straight line was obtained when the same was



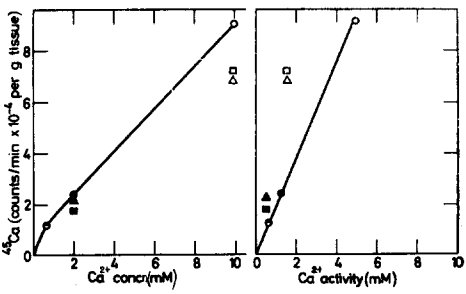




Fig. 2. The accumulation of $^{45}Ca^{2+}$ as a function of $Ca^{2+}$ concentration (●—●) and $Ca^{2+}$ activity (○—○). The specific activity of the medium was $4.0 \cdot 10^7$ counts/min per mmole $^{40}Ca^{2+}$.

Fig. 3. The effect of Na-EDTA and sodium citrate on $^{45}Ca$ accumulation. ○, 10.0 mM $Ca^{2+}$; ●, 2.0 mM $Ca^{2+}$; ◒, 0.6 mM $Ca^{2+}$; □, 10.0 mM $Ca^{2+}$ and 4.3 mM Na-EDTA; ■, 2.0 mM $Ca^{2+}$ and 1.3 mM Na-EDTA; △, 10.0 mM $Ca^{2+}$ and 9.6 mM sodium citrate; ▲, 2.0 mM $Ca^{2+}$ and 2.5 mM sodium citrate. The specific activity of the medium was $5.0 \cdot 10^7$ counts/min per mmole $^{40}Ca^{2+}$.

taken as a function of the $Ca^{2+}$ activity (Fig. 2). A statistical test for linearity indicated the first but not the second relationship to be significantly ($P<0.01$) different from the linear one. Similar results were obtained in three other experiments.

In an experiment in which the $Ca^{2+}$ activity was reduced by chelating agents, jejunal slices were incubated in media containing 0.6, 2.0 or 10.0 mM $Ca^{2+}$. In additional solutions containing either 2.0 or 10.0 mM $Ca^{2+}$, $Ca^{2+}$ activity was reduced by the addition of EDTA (sodium salt) or sodium citrate, and the $^{45}Ca^{2+}$ accumulation was measured as a function of the $Ca^{2+}$ concentration or of the $Ca^{2+}$ activity.

From Fig. 3 it is apparent that both chelating agents reduced the $^{45}Ca$ accumulation when the latter was related to the $Ca^{2+}$ concentration of the medium. However, when the $^{45}Ca$ accumulation was expressed as a function of the $Ca^{2+}$ activity, accumulation in the presence of the chelating agent was higher than occurred with the same $Ca^{2+}$ activity but without the agents.

*The effect of $Na^+$ on $^{45}Ca^{2+}$ accumulation*

*Series 1.* The kinetics of $^{45}Ca^{2+}$ accumulation from a medium poor in $Na^+$ was investigated in a series of experiments with slices from the upper jejunum. The accumulation of $^{45}Ca^{2+}$ as a function of $Ca^{2+}$ activity in the medium appeared to be linear at both $Na^+$ concentrations of 5.0 and 125.0 mM, but the slope was considerably greater in the medium poor in $Na^+$ (Fig. 4). In 17 other experiments in which 110–125 mM $Na^+$ were replaced by mannitol, the $^{45}Ca^{2+}$ accumulation was increased by $56 \pm 23\%$, and in 10 experiments by $38 \pm 23\%$ in media containing 2.0 or 10.0 mM $Ca^{2+}$, respectively.

*Series 2.* Since in the first series whole intestinal slices were used, it was of importance to determine which of the intestinal surfaces, mucosal or serosal, responded to the changes in the environmental $Na^+$ concentration. Jejunal loops were incubated in media containing 2.0 mM $Ca^{2+}$ and either 5 or 125 mM $Na^+$ ($Na^+$-poor and -rich media, respectively). $^{45}Ca^{2+}$ was added to either the mucosal or the serosal bath. In one experiment, the entire intestinal tissue was analyzed for $^{45}Ca^{2+}$, while in another the mucosa and the underlying tissues were analyzed separately.

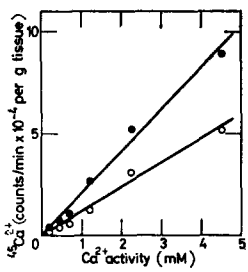



Fig. 4. $^{45}Ca^{2+}$ accumulation by intestinal tissue as a function of $Ca^{2+}$ activity, for solutions with either high (○—○) or low (●—●) $Na^+$ concentrations. The specific activity of the $Ca^{2+}$ in the media was $4.0 \cdot 10^7$ counts/min per mmole $^{40}Ca^{2+}$.

Results given in Table II indicate that the effect of $Na^+$ deficiency on the accumulation of $^{45}Ca^{2+}$ by the entire tissue was greater for loops incubated in a labeled mucosal than in a labeled serosal bath (78% *versus* 35%). The corresponding increase in accumulation by the mucosal tissue was 102% as compared with 63% in the underlying tissues.

TABLE II

PARTITION OF THE "LOW $Na^+$ EFFECT" ON $^{45}Ca^{2+}$ ACCUMULATION BETWEEN THE MUCOSA AND THE SEROSA

$Na^+$ was varied at the expense of mannitol. The number of birds is given in parentheses.

| *Trial No.* | *Tissue* | *Increase (%) in $^{45}Ca^{2+}$ accumulation** | |
|---|---|---|---|
| | *Radioisotope in:* | *Mucosal medium* | *Serosal medium* |
| 1 | Whole | 78.0 (5) | 34.8 (5) |
| 2 | Mucosa | 102.0 (7) | −8.2 (7) |
| | Underlying tissues | −6.2 (7) | 63.0 (7) |

* $100 \times (^{45}Ca_l^{2+} - ^{45}Ca_h^{2+})/^{45}Ca_h^{2+}$. $^{45}Ca_l^{2+}$ and $^{45}Ca_h^{2+}$ designate the $^{45}Ca^{2+}$ accumulation from $Na^+$-poor and -rich media, respectively.

With our technique, the accumulation is usually related to the final fresh weight. It was therefore important to determine if the $Na^+$ effect was not merely the reflection of the accumulation of water by the tissue.

Jejunal slices were incubated in media containing either 5 or 125 mM $Na^+$, 2.0 mM $CaCl_2$ and $10^8$ counts/min per l $^{45}Ca^{2+}$. The results presented in Table III indicate that the accumulation of fluid by the mucosa was higher ($P<0.05$) in the media containing high $Na^+$ than in the one containing little $Na^+$. Although calculating the $^{45}Ca^{2+}$ accumulation on the basis of initial weight slightly minimized the response to $Na^+$, the effect was still preserved.

TABLE III

EFFECT OF $Na^+$ ON WATER AND $^{45}Ca^{2+}$ ACCUMULATION BY SEPARATED INTESTINAL MUCOSA*

$Na^+$ was varied at the expense of mannitol. $^{45}Ca^{2+}$ concentration was $10^8$ counts/min per l.

| | *$Na^+$ concn. (mM)* | | |
|---|---|---|---|
| | *5* | *125* | *S.E.* |
| Water accumulation, $\mu$g/g | 72* | 154* | 45* |
| $^{45}Ca^{2+}$, counts/min $\times 10^{-3}$ per g final wt. | 34.9** | 23.1** | 2.4** |
| $^{45}Ca^{2+}$, counts/min $\times 10^{-3}$ per g initial wt. | 37.5** | 27.3** | 3.5** |

* Initial water concentration was 818 $\mu$g/g fresh tissue. Average from 8 slices.
** Value is the average of 12 observations.

*Series 3.* The transport of $Na^+$ is known to be inhibited by cardiac glycosides such as ouabain[21–23] and to be enhanced by actively transported sugars[24,25]. The effect of the actively transported sugars on $Na^+$ transport is inhibited by phlorizin[25].

If the $Na^+$ pump could serve $Ca^{2+}$ in the absence of this monovalent cation, the $Ca^{2+}$ transport under conditions of $Na^+$ deficiency may be expected to be inhibited by one or both inhibitors and to be sugar dependent. In order to test this hypothesis, jejunal slices were incubated in media containing 2.0 mM $CaCl_2$ and either 5 or 125 mM NaCl. The media contained either glucose or fructose with or without 0.5 mM phlorizin or 0.1 mM ouabain.

In the media containing large amounts of $Na^+$, $^{45}Ca^{2+}$ accumulation was significantly ($P<0.05$) higher for slices incubated in media containing fructose than in those containing glucose. Such a stimulation of $^{45}Ca^{2+}$ accumulation was not observed with media poor in $Na^+$ (Table IV).

TABLE IV

EFFECT OF INHIBITORS ON $^{45}Ca^{2+}$ ACCUMULATION FROM MEDIA WITH LOW AND HIGH $Na^+$ CONCENTRATIONS

$Na^+$ was varied at the expense of mannitol. Each value is the average from several experiments ± S.D. The number of experiments is given in parentheses. In any one experiment, each treatment was applied to 12 slices taken from 4 birds.

| *Inhibitor* | *Concn. (mM)* | *Sugar (20.0 mM)* | $^{45}Ca^{2+}$ *accumulation (% of control)** | |
|---|---|---|---|---|
| | | | *125 mM* $Na^+$ | *5 mM* $Na^+$ |
| None | — | Glucose | 83 ± 7 (4) | 106 (2) |
| Ouabain | 0.1 | Fructose | 88 ± 5 (5) | 101 (2) |
| | | Glucose | 78 (1) | 91 (1) |
| Phlorizin | 0.5 | Fructose | 104 (2) | 99 (2) |
| | | Glucose | 98 (1) | 102 (1) |

* For each $Na^+$ level; the control media contained 20 mM fructose.

Ouabain inhibited $^{45}Ca^{2+}$ accumulation significantly ($P<0.05$) in two out of five experiments with the media rich in $Na^+$ and fructose, and in one experiment with media rich in $Na^+$ with glucose ($P<0.01$). This inhibitor did not significantly ($P>0.05$) influence the accumulation of $^{45}Ca^{2+}$ in media poor in $Na^+$. Similar results were obtained for separated mucosa (unpublished data). Phlorizin at 0.5 mM did not influence $^{45}Ca^{2+}$ accumulation significantly ($P>0.05$) in either media.

*Series 4.* It seemed reasonable to assume that incubation in a medium poor in $Na^+$ would bring about various metabolic changes in the tissue which, in turn, could influence the accumulation of $Ca^{2+}$ either directly[18] or through irreversible damage to the tissue. Under the latter conditions, the mucosal cells might not act as a permeability barrier.

In order to test the effect of $Na^+$ in the medium on the oxidative metabolism, $O_2$ consumption by either intestinal slices or separated mucosa was measured.

The tissue samples were incubated in a Warburg apparatus under normal atmosphere and at 39° in 3 ml of isotonic media which contained in mM: 2.0 $CaCl_2$, 0.4 $MgSO_4$, 3.0 KCl, 3.0 phosphate buffer (pH 6.8), 5.0 or 125.0 NaCl, varied at the expense of mannitol.

Results presented in Fig. 5 indicate that during 120 min of incubation, the $O_2$ consumption by either slices or by separated mucosa was higher in the medium rich in $Na^+$ than in the one poor in $Na^+$.

In order to test whether the effect of the low $Na^+$ treatment was reversible, slices were preincubated 30 min in media rich in or poor in $Na^+$. Some of the slices preincubated in the medium poor in $Na^+$ were washed and incubated 90 min in fresh medium rich in $Na^+$ (low–high treatment). The rest were washed and incubated 90 min in a medium poor in $Na^+$ (low–low treatment). The slices which were preincubated

in the medium rich in $Na^+$ were washed and incubated in the same type of fresh medium (high–high treatment).

Results presented in Fig. 6 demonstrate the dependence of $O_2$ consumption on the presence of high $Na^+$ concentrations. There was hardly any difference between the low–high and high–high treatments, indicating a decided degree of reversibility.

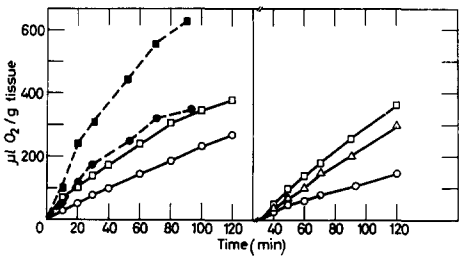



Fig. 5. $O_2$ consumption by jejunal tissue as influenced by the $Na^+$ concentration of the medium. Each value is the average of 6 samples. □—□, slices (high $Na^+$); ○—○, slices (low $Na^+$); ■---■, mucosa (high $Na^+$); ●---●, mucosa (low $Na^+$).

Fig. 6. The dependence of $O_2$ consumption by jejunal slices on preincubation in media containing, high or low $Na^+$ concentration. Each value is the average from 4 slices taken from 4 birds. □—□ high–high treatment; △—△, low–high treatment; ○—○, low–low treatment. For full details of treatments, see the text.

The reversibility of the effect of incubation in media poor in $Na^+$ on $^{45}Ca^{2+}$ accumulation was tested with jejunal slices. The experimental design was the same as in the previous experiment (Fig. 6).

The $^{45}Ca^{2+}$ concentration of the tissues in counts/min $\times 10^{-3}$ per g was 15.3 for the high–high treatment, 15.0 for the low–high treatment and 25.1 for the low–low treatment. It is apparent that the effect due to $Na^+$ deficiency was almost completely reversible.

## DISCUSSION

The accumulation of $^{45}Ca^{2+}$ by the laying hen intestinal slices was linearly related to the $Ca^{2+}$ activity in the medium when no chelating agents were present (Fig. 2). Those results are different from those of SCHACHTER *et al.*[3] which indicated saturation kinetics with $^{45}Ca^{2+}$ accumulation by rat duodenal slices. The apparent disagreement between their results and ours may be due to the following reasons: (a) those authors related the transport to concentration rather than to activity; (b) the concentrations used by SCHACHTER *et al.*[3] were lower than 2.0 mM, and (c) different species and intestinal segments were used. The linear relationship between the $Ca^{2+}$ accumulation and the $Ca^{2+}$ activity would be indicative of a diffusion process; it does not eliminate the possibility of a carrier-mediated transport when the carrier is far from saturation[26]. Other kinetic evidence indicates that the $Ca^{2+}$ concentration in the medium required to maintain the tissue $Ca^{2+}$ is similar to the tissue $Ca^{2+}$ concentration (Fig. 1).

The kinetic evidence in addition to the relatively slight response to metabolic inhibitors, $O_2$ and temperature[20], suggests that the $Ca^{2+}$ transport into the intestinal wall of the laying hen is a simple diffusion process.

The mucosal transport of $^{45}Ca^{2+}$ out of media poor in $Na^+$ was considerably higher than from media rich in $Na^+$. This observation is in accordance with that of HARRISON AND HARRISON[12] and of HURWITZ *et al.*[13].

According to SCHACHTER[18], the permeability barrier for $Ca^{2+}$ in the rat jejunal and ileal mucosa is dependent on $O_2$. Accordingly, the stimulation of $^{45}Ca^{2+}$ accumulation by low $Na^+$ concentration could result from the decrease in $O_2$ consumption. However, we previously observed a small decrease in $^{45}Ca^{2+}$ accumulation under anaerobic conditions and with 2,4-dinitrophenol[20]. Thus, the decrease in oxidative metabolism did not diminish the permeability barrier for $Ca^{2+}$ in our system, and the effect of $Na^+$ deficiency cannot be explained on this basis. Furthermore, no irreversible changes due to the $Na^+$ deficiency were observed in the respiratory activity or in $^{45}Ca^{2+}$ accumulation. Therefore the enhanced $Ca^{2+}$ accumulation under such conditions could not result from irreversible tissue damage ("death").

The accumulation of $^{45}Ca^{2+}$ during $Na^+$ deficiency was sensitive neither to glucose nor to phlorizin and ouabain. Furthermore, the function of $Ca^{2+}$ accumulation *versus* media $Ca^{2+}$ activity under these conditions was linear as was the case with the corresponding function under conditions of high $Na^+$ concentrations. The present results, therefore, do not indicate any alternative mechanism to simple diffusion under conditions of $Na^+$ deficiency.

The suggested $Na^+$–$Ca^{2+}$ relationship refers only to the transport into the mucosa. Additional factors may contribute to the inhibitory effect of $Na^+$ on the transmural transport of $Ca^{2+}$, which was observed by HARRISON AND HARRISON[12] and by HURWITZ *et al.*[13].

In agreement with the results obtained by SANUI AND PACE[27] with rat liver microsomes and by VAN'T KLOOSTER AND CARE[28] with sheep intestine, the chelating agent reduced the accumulation of $^{45}Ca$ by the intestine (Fig. 2). However, the accumulation remained considerably higher than expected by the corresponding decrease in $Ca^{2+}$ activity of the medium. This may suggest some transport of $Ca^{2+}$ into the intestinal wall in the chelated form similar to the transport of iron[29] and zinc[30].

The transport of bound Ca may be of considerable importance in the absorption of this mineral *in vivo*, since we have demonstrated the presence of such $Ca^{2+}$ in the intestinal lumen[10].

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