

Study on Active Transport of Calcium in Laying Hens

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THIS experiment was carried out at Poultry Research Farm belonging to Animal Production Department, Faculty of Agriculture, Zagazig University, Benha Branch.

The objectives of this work were to detect the nature of active transport of calcium through the different regions of the intestinal tract as well as to study some factors affecting it.

A total number of 49 laying Matrouh hens aged 80 weeks were used. All birds were fed *ad lib* on balanced laying ration containing 3.7% calcium and 0.7% phosphorus.

Hens were starved for 24 hr prior to experiment and killed by a blow on the head 3 hours after oviposition. The abdomen was opened and small intestine was removed and divided into its different regions (duodenum, jejunum and ileum).

Open-gut sac method was applied (according to Carne and Wilson, 1958) to study the active transport of calcium in the different intestinal regions during 120 minutes of incubation at 39°C using two calcium chloride levels (0.002 M and 0.003 M) in the incubating medium.

Closed-gut sac method was used (according to Medge, 1975) for detecting active transport of calcium in the different intestinal regions under the influence of substrate content, oxygen supply, incubating temperature, oxidative metabolism inhibitors, oxidative phosphorylation inhibitors, divalent cations and calcium chelating compounds.

Active transport of calcium was expressed as the inside/outside concentration ratio.

Results obtained could be summarized as follows :

- 1) The rate of active transport of calcium through all intestinal parts decreased as its concentration in the incubating medium increased.
- 2) The highest calcium concentration ratios were observed in the duodenum and progressively lower ratios were obtained in the more distal segments.

- 3) Using glucose as substrate, supplying oxygen to the incubating medium and rising the incubating temperature increased the rate of calcium active transport measured as calcium concentration ratio.
- 4) Applying oxidative metabolism inhibitors, oxidative phosphorylation inhibitors and calcium — chelating compounds resulted in decreasing the rate of active transport of calcium. On the other hand, divalent cations did not show any significant effect.
- 5) In all cases, the response to any treatment applied was the highest in duodenum than the two other intestinal segments (jejunum and ileum).

It is well known that calcium is considered as a very important element needed for chickens along the growing period up to sexual maturity for the formation of the skeletal system. In addition it has a pronounced importance throughout the laying period for the formation of the egg shell. Many investigations had been conducted to find out the hormonal role on calcium mobilization from bones to blood during the time of shell formation. However, it is now well stated that the major portion of calcium needed for shell deposition comes from the food (Sturkie, 1976) which mainly depends on the rate of calcium active transport throughout the intestinal tract (Hurwitz and Bar, 1965).

Calcium absorption had been thoroughly studied either in intact organism (Cohen, *et al.*, 1978 and Hurwitz, *et al.*, 1973) or in isolated intestinal loops (Bar and Hurwitz, 1969, Chow *et al.*, 1972 and El-Habbak and Radwan, 1984).

The active transport of calcium from the mucosal to the serosal surfaces of the small intestine was found to require energy elaborated from either the oxidative metabolic processes or the generation of phosphate-bond energy in the intestine (Martin and DeLuca, 1969, Papworth and Patrick, 1970 and Caspary, 1972).

Several factors have been implicated in intestinal calcium transport : a calcium binding protein an exchange of calcium for sodium, calcium transport into mitochondria, and the intervention of the enzymes alkaline phosphatase and calcium-sensitive ATPase (McCuaig *et al.*, 1972 and Bonjour *et al.*, 1973).

The nature of active transport of calcium through the intestinal tract of chicken is not yet fully established. So, we aimed to study some factors affecting it.

Material and Methods

This experiment was conducted at Poultry Research Farm belonging to Animal Production Department, Faculty of Agriculture, Zagazig University, Benha branch.

A total of 49 laying Matrouh hens aged 80 weeks were used. All birds were fed *ad lib* on balanced laying ration containing 3.7% calcium and 0.7% phosphorus and kept at standard and similar hygienic and environmental conditions.

Hens for each estimation were starved for 24 hr prior to experiment and killed, by a blow on the head, 3 hr after oviposition (Bar and Hurwitz, 1969). Then abdomen was opened and the small intestine was carefully removed and divided into its various regions (Duodenum, jejunum and ileum).

To investigate the effect of the different factors on active transport of calcium, two types of gut sacs were prepared from each intestinal region :

1. *Opened gut-sac*

This was applied in studying the active calcium transportation in the different intestinal regions as affected by time of incubation and calcium concentration in the incubating medium using the method recommended by Carne and Wilson, (1958) with slight modification. Small intestine was segmented into duodenum, upper and lower jejunum and upper and lower ileum. Each segment was everted and closed with ligature. The inside was filled with 5-10 ml of medium (according to the diameter of segment). The open end was fastened around a cannula and the segment was suspended in 200 ml of the same medium in a large plastic tube. Two media were used. Both contained 0.135 M NaCl; 0.011 M KCl; 0.02 M glucose and 0.008 M sodium phosphate. In addition the first medium contained 0.002 M CaCl₂ while the second contained 0.003 M CaCl₂. Oxygen was bubbled continuously into the outer medium via a plastic catheter and the temperature was maintained at 39°C in a water bath for 2 hrs.

Samples of 0.2 ml from the inner and outer media were taken at 30, 60, 90, and 120 minutes for estimation of calcium concentration in each media applying EDTA titration (Hawks, 1965).

2. *Closed gut-sac*

This was applied according to Madge (1975) for detecting active calcium transport in the intestinal tract under the influence of the following factors : Substrate content, oxygen supply, incubating temperature, oxidative metabolism, oxidative phosphorylation, inhibitors, divalent cations and calcium-chelating compounds.

Two gut-sacs, prepared from each intestinal region, were filled with 5 ml of standard medium and ligatured at two ends. One sac was incubated in the standard medium (control), while the other was incubated in the same medium plus the tested factor as shown in the following Table.

<i>Tested factor</i>	<i>Standard medium</i>	<i>Medium + tested factor</i>
Glucose	Free	0.02 mol.
Oxygen supply	No oxygen	oxygen supplied
Incubating temperature	39°C	5°C
Sodium fluoride (1)	Free	0.002 mol.
Mercuric chloride (1)	Free	0.001 mol
2, 4-dinitrophenol (2)	Free	0.00005 mol
Magnesium chloride (3)	Free	0.001 mol
Barium chloride (3)	Free	0.001 mol
Sodium versenate (4)	Free	0.02 mol

1, 2, 3 and 4 were used as oxidative metabolism inhibitors, oxidative phosphorylation inhibitor, divalent cations and calcium-chelating compounds, respectively.

All media were supplied 0.003 mol calcium chloride. After the end of the incubating period (30 minutes), samples of 0.2 ml were taken from the inner and the outer media to determine calcium concentration in each of them applying the same method mentioned before. Active transport of calcium was expressed as inside/outside concentration ratio.

Statistical analysis was carried out according to Snedecor and Cochran, (1967). The significance of differences were tested by the use of LSD.

Results and Discussion

1. Dynamic of active calcium transport in various intestinal regions as influenced by calcium concentration in the incubating medium

Data concerning the effect of calcium concentration level in the incubating medium on the rate of active calcium transport are listed in Table 1 and graphically illustrated in Fig. 1. Inspection of data obtained showed that the rate of calcium active transport decreased as the concentration of calcium in the media increased. This was quite true for all intestinal segments studied except the lower part of the ileum which showed maximum transpotation rate

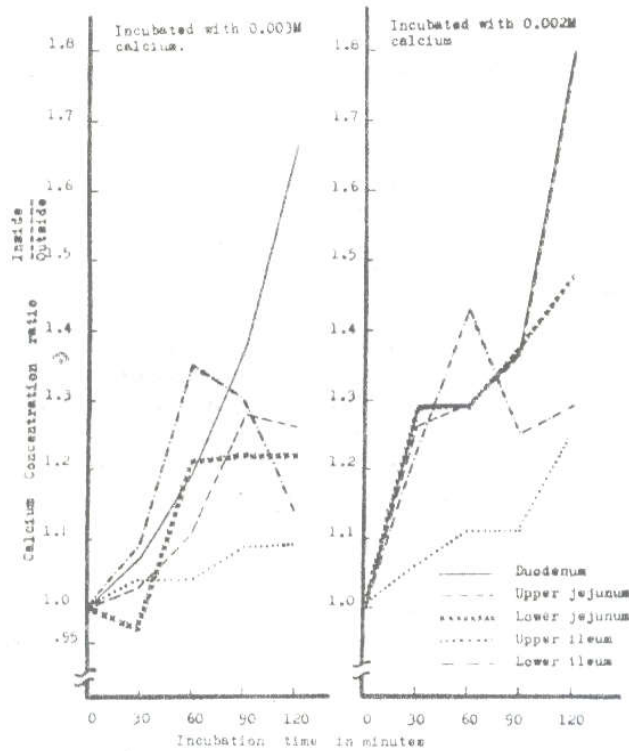


Fig. (1) : Dynamic of active calcium transport in various intestinal regions as influenced by calcium concentration in the incubating medium.

at 60 min then it started to decline. When the open gut sacs were incubated in media containing 0.003 M and 0.002 M calcium for 120 min at 39°, the final calcium concentration ratios (inside / outside) averaged 1.67 and 1.80 in duodenum, 1.26 and 1.79 in upper jejunum, 1.22 and 1.48 in lower jejunum, 1.09 and 1.25 in upper ileum and 1.14 and 1.29 in the lower ileum, respectively. Variations in the rate of calcium absorption due to the calcium concentration in the incubating medium were significant ($P < 0.01$) (Table 2).

Table (1) : Dynamic of active calcium transport in various intestinal regions as influenced by calcium concentration.

Intestinal region	Time of incubation (min)	Calcium concentration ratio (Inside/Outside)			
		$\bar{x} \pm S.D.$			
		30	60	90	120
Incubated with 0.002 M calcium					
Duodenum		1.29±0.11	1.29±0.16	1.36±0.14	1.80±0.14
Upper jejunum		1.26±0.17	1.29±0.21	1.37±0.14	1.79±0.22
Lower jejunum		1.29±0.23	1.29±0.10	1.36±0.14	1.48±0.17
Upper ileum		1.06±0.05	1.11±0.07	1.11±0.11	1.25±0.06
Lower ileum		1.12±0.13	1.43±0.20	1.25±0.26	1.29±0.07
Incubated with 0.003 M calcium					
Duodenum		1.07±0.09	1.19±0.05	1.38±0.12	1.67±0.13
Upper jejunum		1.03±0.22	1.11±0.07	1.28±0.18	1.26±0.23
Lower jejunum		0.97±0.16	1.21±0.09	1.22±0.09	1.22±0.15
Upper ileum		1.04±0.05	1.04±0.05	1.09±0.03	1.09±0.24
Lower ileum		1.09±0.06	1.35±0.22	1.30±0.16	1.14±0.11

Each value represents mean \pm SE for 4 laying hens

Results also indicated that calcium concentration ratios varied significantly ($P < 0.01$) according to the small intestinal segment examined (Table 2). The highest ratios were observed in the duodenum and progressively lower ratios were obtained in the more distal segments.

Rate of calcium absorption, of all intestinal segments, increased as the time of incubation period increased. The rate of increase varied according to the intestinal segment. This was

emphasized by the significant effect ($P < 0.01$) of incubation time and the interaction between this factor and the intestinal segment on the rate of calcium absorption. (Table 2).

Table (2) : Analysis of variance for Table 1.

Source of variation	D.F.	Mean squares
Between calcium concentration ratio(C)1	1	0.7728**
Between intestinal region(R)	4	0.2057**
Between time of incubation (T)	3	0.5257**
Interaction(CxR)	4	0.0581
Interaction(CxT)	3	0.0749
Interaction(RxT)	12	0.1466**
Interaction(CxRxT)	12	0.0167
Error	120	0.0484

** $P < 0.01$

L.S.D. values for

	0.05	0.01
Intestinal region	0.11	0.14
Time of incubation	0.10	0.13

II. Effect of factors studied on the rate of active transport of calcium

Data concerning calcium concentration ratio under the influence of various factors tested are listed in Table 3.

1. *Substrate content* It was obviously clear that the existence of glucose at the rate of 0.02 M in the incubating medium resulted in increasing the calcium concentration ratio indicating the active transport of calcium. This was quite true in all intestinal regions. The rate of increase was higher in duodenum (0.83) and exactly the same in both jejunum and ileum (0.28). Analysis of variance indicated significant variation in calcium concentration ratio due to either treatment or intestinal region ($P < 0.01$). In addition significant interaction effect between treatment and intestinal regions was also found ($P < 0.01$) (Table 4).

Table (3) : Factors affecting active transport of calcium by everted gut sac from different small intestinal regions.

Factors studied	Calcium concentration ratio (Inside/Outside). $\bar{x} \pm$ S.D.		
	Duodenum	Jejunum	Ileum
1. Substrate:			
Glucose(0.02M)	2.26 \pm 0.09	1.48 \pm 0.07	1.41 \pm 0.10
No substrate	1.43 \pm 0.05	1.20 \pm 0.08	1.13 \pm 0.07
2. Oxygen supply			
With	1.19 \pm 0.07	1.13 \pm 0.12	1.13 \pm 0.11
Without	1.00 \pm 0.01	0.98 \pm 0.03	0.99 \pm 0.02
3. Incubating temperature			
At 5°C	1.05 \pm 0.28	1.57 \pm 0.12	1.79 \pm 0.19
At 39°C	2.10 \pm 0.16	2.00 \pm 0.24	2.02 \pm 0.24
4. Oxidative metabolism inhibitors			
Sodium fluoride(0.002M)	0.98 \pm 0.05	1.01 \pm 0.05	0.98 \pm 0.03
No sodium fluoride	1.27 \pm 0.04	1.19 \pm 0.04	1.17 \pm 0.04
Mercuric chloride(0.001M)	1.03 \pm 0.05	1.05 \pm 0.05	1.02 \pm 0.03
No mercuric chloride	1.25 \pm 0.08	1.19 \pm 0.07	1.19 \pm 0.10
5. Oxidative phosphorylation inhibitors			
2,4-dinitrophenol(0.00005M)	1.02 \pm 0.05	1.01 \pm 0.05	0.96 \pm 0.02
No 2,4-dinitrophenol	1.36 \pm 0.09	1.24 \pm 0.05	1.14 \pm 0.11
6. Divalent cations			
Mg Cl ₂ (0.001M)	1.24 \pm 0.03	1.19 \pm 0.06	1.14 \pm 0.04
No Mg Cl ₂	1.24 \pm 0.03	1.17 \pm 0.03	1.17 \pm 0.05
Ba Cl ₂ (0.001M)	1.83 \pm 0.14	1.53 \pm 0.11	1.50 \pm 0.09
No Ba Cl ₂	1.85 \pm 0.08	1.45 \pm 0.10	1.45 \pm 0.14
7. Calcium-chelating compound			
Sodium versenate(0.02M)	1.05 \pm 0.03	1.00 \pm 0.02	0.98 \pm 0.02
No sodium versenate	1.38 \pm 0.08	1.22 \pm 0.05	1.23 \pm 0.05

Each value represents mean \pm SE for 5 laying hens.

2. *Oxygen supply* : It was found that calcium concentration ratio (inside/outside) was higher, in all intestinal region, when oxygen was supplied during the incubation period. Analysis of variance showed significant effect of oxygen supply ($P < 0.01$) on calcium concentration ratio (Table 4). The response of duodenum seemed to be higher when compared with either jejunum or ileum. However variation in calcium concentration ratio due to intestinal part was found to be of no significant value (Table 4). Obtained results indicate that intestinal tissues permitted to undergo oxida-

tive metabolism took up more calcium from the outer medium and actively transfer it to the inner medium. These results are supported with several investigators who demonstrated that calcium uptake requires oxygen. This indicates that active calcium transport is energy dependent process. (Bar and Hurwitz, 1969 ; Martin and DeLuca, 1969 ; Papworth and Patrick, 1970 and Caspary, 1972).

Table (4) : Analysis of variance for table 3.

Items	Source of variation	D.F.	Mean squares
Substrate (Glucose)	Between treatment(T)	1	1.619 ^{**}
	Between regions(R)	2	0.984 ^{**}
	Interaction(TxR)	2	0.248 ^{**}
	Error	24	0.006
Oxygen supply	Between treatment(T)	1	0.197 ^{**}
	Between regions(R)	2	0.004
	Interaction(TxR)	2	0.002
	Error	24	0.006
Incubating temperature	Between treatment(T)	1	2.465 ^{**}
	Between regions(R)	2	0.270 [*]
	Interaction(TxR)	2	0.460 ^{**}
	Error	24	0.070
Sodium fluoride	Between treatment(T)	1	0.370 ^{**}
	Between regions(R)	2	0.006
	Interaction(TxR)	2	0.008 [*]
	Error	24	0.002
Mercuric chloride	Between treatment(T)	1	0.218 ^{**}
	Between regions(R)	2	0.004
	Interaction(TxR)	2	0.005
	Error	24	0.004
2,4-dinitrophenol	Between treatment(T)	1	0.444 ^{**}
	Between regions(R)	2	0.040 ^{**}
	Interaction(TxR)	2	0.022 ^{**}
	Error	24	0.005
Mg Cl ₂	Between treatment(T)	1	0.0001
	Between regions(R)	2	0.0186 ^{**}
	Interaction(TxR)	2	0.0013
	Error	24	0.0017

Table (4) : (Continued)

Items	Source of variation	D.F.	Mean squares
Ba Cl ₂	Between treatment(T)	1	0.377
	Between regions(R)	2	0.410**
	Interaction(TxR)	2	0.005
	Error	24	0.013
Sodium versenate	Between treatment(T)	1	0.517**
	Between regions(R)	2	0.038**
	Interaction(TxR)	2	0.007
	Error	24	0.003

** P < 0.01

* P < 0.05

L.S.D. values for the intestinal regions :

	5%	1%
Substrate (glucose)	0.07	0.10
Incubating temperature	0.25	0.34
2'4-dinitrophenol	0.06	0.09
MgCl ₂	0.04	0.05
BaCl ₂	0.10	0.14
Sodium versenate	0.05	0.07

3. *Incubating temperature* : Calcium concentration ratio increased as incubating temperature increased (Table 3). Incubating temperature was found to have a significant effect ($P < 0.01$) on the rate of active calcium transport.

The rate of increase was high in duodenum (1.05), low in ileum (0.23) and intermediate in jejunum (0.43) (Table 3). Analysis of variance (Table 4) showed a significant variation ($P < 0.05$) in calcium concentration ratio due to the intestinal parts as well as due to the interaction between treatment and intestinal parts ($P < 0.01$) (Table 4).

4. *Oxidative metabolism inhibitor* : Sodium fluoride (0.002 M) and mercuric chloride (0.001 M) were used as oxidative metabolism inhibitors. It was found that the rate of calcium active transport decreased when any of the two materials was applied. The greatest effect was found due to the sodium fluoride, while mer-

curic chloride had relatively slight effect on decreasing the calcium concentration ratio. Analysis of variance showed significant effect ($P < 0.01$) of both treatments on calcium concentration ratio. (Table 4).

On the other hand, the response to any material differed according to the intestinal region. The higher response to the oxidative metabolism inhibiting material applied was found in duodenum, followed by that in ileum and jejunum. Calcium concentration ratio decreased by 0.29 and 0.22 in duodenum, 0.18 and 0.14 in jejunum and 0.19 and 0.17 in ileum when applying sodium fluoride and mueric chloride respectively (Table 3). However, variation in calcium concentration ratio due to the intestinal part was not significant (Table 4).

5. *Oxidative phosphorylation inhibitors* : Applying 2, 4 dinitrophenol (0.0005 M) as an oxidative phosphorylation inhibitor resulted in decreasing the calcium concentration ratio. This was quite true in all intestinal regions. However, the rate of decrease was relatively higher in duodenum (0.34) and lower in iluem (0.16) when compared with jejunum (0.23). (Table 3). Statistical analysis of data revealed that variations in calcium concentration ratio due to treatment, intestinal part and the interaction between them were significant ($P < 0.01$) (Table 4).

6. *Divalent cations* : Magnesium and barium chlorides were added to the incubating medium to study the effect of divalent cations on the active calcium transport. Inspection of data obtained (Table 3) showed that the effect of the two divalent cations applied on the concentration ratio were not similar and had no characteristic trend. In addition, analysis of variance for data obtained (Table 4) showed no significant effect due to treatments applied on calcium concentration ratio. However, intestinal regions showed significant different response ($P < 0.01$) to the divalent cations applied (Table 4).

7. *Calcium-chelating compound* : Calcium concentration ratio decreased under the influence of sodium versenate (applied as calcium-chelating compound). Variation in this trait due to treatment applied was significant ($P < 0.01$) (Table 4).

The rate of increase was relatively higher in duodenum (0.33) and lower in jejunum (0.22) when compared with ileum (0.25). Analysis of variance revealed significant variation ($P < 0.01$) in calcium concentration ratio due to intestinal region (Table 4).

Studying factors affecting the rate of active calcium transport (estimated as calcium concentration ratio between outside and inside media) showed significant effect of glucose (as substrate), oxygen supply, incubating temperature, oxidative phosphorylation inhibitors and calcium-chelating compounds. On the other hand, the two divalent cations had no significant effect.

Vitro studies with everted gut sac (Morz, 1972 and Eiwe, 1972) showed that magnesium ions inhibits the active transport of calcium ions (Alcock and MacIntyre, 1962 and Clark, 1969). It was stated that there is a common absorptive site for calcium and magnesium in the intestine (Clark, 1969) for which these ions compete. Further evidence for the competition between calcium and magnesium ions was obtained from the studies of Alcock and MacIntyre, (1962) who found that calcium absorption in the intestine was significantly enhanced when diet was free of magnesium. They suggested that the presence of magnesium ions inhibits calcium absorption. They added that in the absence of dietary calcium, magnesium appeared to stimulate parathyroid function. These results disagree with those of the present study which may be due to the short incubation time applied.

Calcium uptake by the intestinal cells seems to include the energy-dependent accumulation of calcium into mitochondria and it may not be a true reflection of calcium transfer across the mucosal plasma membrane alone. The dependence of calcium uptake on energy metabolism may be a function of the substrate available, of ATP levels of the cell and of the kind of inhibitors which may either block electron transport, incouple oxidative phosphorylation, block energy transfer or inhibit calcium efflux of the cell (Adams and Norman, 1970 and Ogata *et al.*, 1971).

Results of the present study agree with the previously mentioned findings and indicate that calcium efflux out of the cell at the serosal side is usually recognized to be an active transport process or at least coupled to metabolically dependent process, since calcium has to move against a chemical potential gradient.

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دراسة الانتقال النشط (الفعال) للكالسيوم في الدجاج الأبيض

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اجرى هذا البحث بمزرعة بحوث الدواجن التابعة لقسم الانتاج الحيواني بكلية الزراعة جامعة الزقازيق - فرع بنها بهدف تعيين طبيعـة الانتقال النشط للكالسيوم خلال مناطق الامعاء الدقيقة المختلفة بالإضافة الى دراسة تأثير بعض العوامل المؤثرة على هذا الانتقال .

استخدمت في هذا البحث ٤٩ دجاجة مطروح بياضة عمر ٨٠ أسبوع . غذيت جميع الدجاجات حتى الشبوع على عليقة متزنة تحتوي على ٢.٧٪ كالسيوم ، ٠.٧٪ فوسفور .

تم تصويم الدجاج لمدة ٢٤ ساعة قبل التجربة وقتلت بضرية على الرأس بعد ثلاثة ساعات من وضعها للبيض . فتح البطن بعد ذلك وازيلت الامعاء الدقيقة ثم تقسيمها الى مناطقها المختلفة (الاثنى عشر - الصائم - اللغائى) .

استخدمت طريقة الكيس المعوى المفتوح (تبعاً لكارن وولسون ١٩٥٨) لدراسة الانتقال النشط للكالسيوم من مناطق الامعاء المختلفة خلال ١٢٠ دقيقة من التحضين على درجة ٣٩°م مستعملين في ذلك تركيزين مختلفين من كلوريد الكالسيوم (٠.٢ ، ٠.٣ ، ٠.٤ ومرل) وذلك في بيئة التحضين . أما طريقة الكيس المعوى المغفول فقد استخدمت (تبعاً لمدج ١٩٧٥) لدراسة معدل الانتقال النشط للكالسيوم خلال مناطق الامعاء المختلفة تحت تأثير اضافة الجلوكوز وامداد الاكسوجين لبيئة التحضين ودرجة حرارة التحضين ومثبطات التمثيل التاكسدى ومثبطات الفسفرة والكاتيونات الشائبة والمركبات الخطافية للكالسيوم .

وتم التعبير عن معدل الانتقال النشط للكالسيوم كنسبة بين تركيزه في البيئة الداخلية الى تركيزه في البيئة الخارجيه .

ويمكن تلخيص النتائج المتحصل عليها فيما يأتى :

- ١ - انخفض معدل الانتقال النشط للكالسيوم لكل مناطق الامعاء بزيادة تركيزه في بيئة التحضين .
- ٢ - كانت أعلى نسبة تركيز للكالسيوم في الاثنى عشر وانخفضت كلما اتجهنا الى الأجزاء السفلى من الامعاء .
- ٣ - ادى اضافة الجلوكوز الى بيئة التحضين وكذا امدادها بالاكسوجين ورفع درجة حرارة التحضين الى زيادة معدل الانتقال النشط للكالسيوم والناس على أساس النسبة بين تركيزه في كل من البيئة الداخلة والخارجية .
- ٤ - ادى استعمال مثبطات التمثيل التاكسدى ومثبطات الفسفرة والمركبات الخطافية الى خفض معدل الانتقال النشط للكالسيوم . أما الكاتيونات الشائبة فلم تظهر أى تأثير معنوى في هذا المجال .
- ٥ - وفي كل الحالات - كانت الاستجابة الى أى معاملة من المعاملات المستعملة عالية في الاثنى عشر عنها في باقى اجزاء الامعاء الدقيقة الأخرى (الصائم - اللغائى) .