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Comparative Studies on the Transmural Potential Difference and the Intensity of the Short-Circuit Current Between the Duodenum and the Ileum of Pigeon

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The transmural potential difference (PD), short circuit current intensity (Isc) and tissue resistance has been studied in duodenum and ileum of pigeon *(Columba livia var domestica)* in media of different ionic compositions.

When the tissue is incubated in a standard solution, the PD across the duodenum is approximately zero, while across the ileum is 7.2 mV (serosa positive with respect to mucosa).

The PD and Isc measurements in duodenum in the absence of sodium on one hand and in the absence of chlorine on the other, reveal the existence of an active sodium absorption from mucosa to serosa, which draws chlorine along in the same direction.

When sodium is substituted in the ileon (Tris as substitute), the PD and the Isc show a marked increase in serosa negativity. In the absence of the chloride ion sulphate as substitute) in ileon the PD and the Isc tend to zero, which indicate that the presence of the ion is necessary for the sodium transport.

Ion transport through epithelial cells has been extensively studied with the short circuit technique which was first used by USSING and ZERAHN (17) for the study of sodium transport across frog skin. Since its introduction this technique has been applied for research on the ion transport in numerous animals on the zoological scale (1, 4, 6, 8, 9).

The mechanisms for ion transport in domestic cock and parrot had already been studied in perfusion experiments *in vivo* (4, 176, 17) and *in vitro* (5, 11, 12). The net transference of sodium from mucosa to serosa revealed the existence of an active absorption of sodium, which triggered other important ionic transferences, such as the net absorption of ammonia and chlorine and the net secretion of potassium.

VÁZQUEZ *et al.* (18) have recently studied the effects of calcium concentration on the transmural potential difference and short circuit current intensity and have concluded that calcium, in the presence of sodium, increases the current potential and intensity in the small intestine of hen much more than it does in chick and rat.

The transmural potential difference and the short circuit current between mucosa and serosa of duodenum and ileum, as well as the variations in such parameters when different ions are substituted in the bath solutions, have been studied herein *in vitro* in dove *Columba livia var. domestica* in order to establish a comparison between the experimental results obtained in the two portions of its small intestine.

Materials and Methods

The pigeon *(Columba livia var. domestica)* specimens were obtained from La Laguna and Santa Cruz de Tenerife, weighed between 350 and 500 g and ranged from 6 to 24 months in age.

After the animals had been anesthetized with ether and their peritoneal sac opened, the duodenum and the ileum were extracted and submerged immediately in Ringer-Tris solution at 0° C. After being washed and everted with tongs, they were inserted into a glass cannula or a U tube similar to the one used by HERRERA (10), in order to measure their transmural potential difference and short circuit current intensity according to the technique described by USSING and ZERAHN (17).

The transmural potential difference was estimated by means of Ringer-agar bridges at 3 %, the ends of which were placed 1 mm from each side of the membrane. The transmural potential difference (PD) between these bridges was measured with a pair of calomel electrodes connected to a Keithley electrometer, mod 600 B of high entrance impedance. The PD across the membrane was expressed in terms of the relation of serosa with mucosa.

The membrane was short-circuited by passing across two pairs of calomel electrodes and agar bridges enough current to reduce the potential difference to zero, while the intensity of the short circuit current (Isc) was measured by means of a microamperimeter inserted in the circuit, and both parameters were recorded every six minutes.

The composition and the millimole concentration of the solutions were as follows: NaCl, 127.27; KCl, 5.09; $CaCl_2$, 2.72; KH_2PO_4, 1.27; $MgSO_4$, 1.27; HCl, 4.10; Tris, 4.9, and glucose, 5.00 mM (pH, 7.2).

When an ion was absent from the solutions the cations were substituted by Tris and the anions by sulphate, except in chlorine and calcium joint solutions, where chlorine was replaced by sulphate, while calcium was eliminated altogether from the medium. Osmolarity was maintained with mannitol. When only chlorine was absent from the solutions, calcium chloride was substituted by calcium sulphate.

The periods of time for all the experiments did not exceed 72 min and the temperature of all the solutions were thermostated at 42° C for the entire experimentation, while their oxygenation was maintained constant at 95 % O_2 and 5 % CO_2.

Results

PD, Isc and tissue resistance in standard solution. The PD values in experiments on duodem in Ringer-Tris solution with every ion are nearly zero and reach a stationary value of —0.5 mv after 24 min of experimentation, remaining constant until the end of the experiment (t = 60 min).

The Isc experienced a parallel pro-

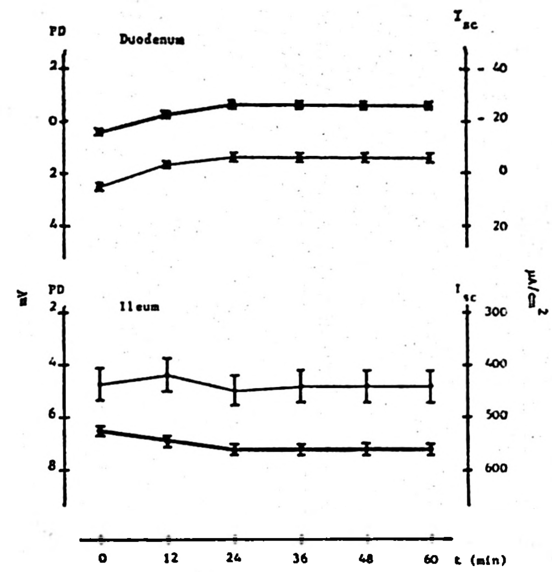

Fig. 1. *Electrical potential difference (PD) and short-circuit current (Isc) across the duodenum and ileum of pigeon.*
▬ PD, —— Isc.

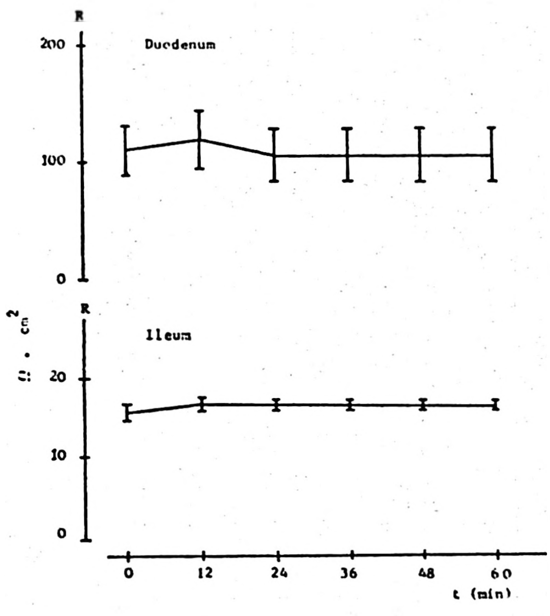

Fig. 2. *Tissue resistance control (R) in duodenum and ileum of pigeon.*

gress and became equally stabilized after 24 min at —6.2 μA cm^{-2} (fig. 1). The tissue resistance deduced from both parameters is 105.1 ohms $\times$ cm^2, without undergoing significant variations during the entire experiment (fig. 2).

In ileum, the PD rose to 7.2 mV (positive serosa) and the Isc to 444.7 μA cm^{-2}. Both parameters reached their stabilization point 24 min after initiation of the experiment, wherefore that period of time was chosen, both for duodenum and ileum, to substitute the medium ions in subsequent trials. The electric resistance in ileum is far inferior to that in duodenum, for it reaches only 16.7 ohms $\times$ cm^2 (fig. 2).

Bicompartimental substitution and restoration of ions in duodenum. A small increase in PD is produced when Na^+ is substituted by Tris on both sides of the duodenum, but it falls back immediately to standard values, i.e. those obtained with that ion in the medium. The same phenomenom occurs with Isc, although the change is less pronounced (table I). These values remain constant until the end of the experiment. Electric resistance increases markedly from standard values to 326.7 ohms $\times$ cm^2 and falls again when sodium is restored to the incubation medium (table I).

When chlorine was eliminated from both sides of the tissue by substituting it with $SO_4^=$, both PD and Isc underwent a remarkable increase which lasted throughout the substitution period (table I). On restoring Cl^- to the medium, however, the standard values reappeared. Tissue resistance also increases with the substitution of chlorine, but the increase is lower than that produced by the absence of sodium ion from the incubation medium.

None of the three parameters underwent any significant changes when the K^+ ion was substituted in the incubation medium.

Table I. *Electrical potential difference (PD), short-circuit current (Isc) and tissue resistance (PD/Isc) across the duodenum of pigeon. Effects of omission and restitution of ions from both compartments.*

Mean values ± S.E. Potential (PD) in mv. Short-circuit current (Isc) in $\mu A/cm^2$ and tissue resistance (PD/Isc) in Ohmios $\times$ cm^2. Number of experiments in parentheses. Changes %: +, increase; —, decrease.

Experimental condition Ion omitted	Param-eters	Time (minutes) and solutions							
		Standard		Omission		Restitution		Changes %	
		0	24	30	48	54	66	Omission	Restitution
Na^+ (6)	PD	+0.7 ± 0.1	+0.1 ± 0.1	+1.8 ± 0.5	—0.3 ± 0.4	—0.6 ± 0.4	+0.4 ± 0.3	—1,730.0[a]	+250.0[d]
	Isc	+12.3 ± 3.1	+1.1 ± 1.3	+11.5 ± 2.1	—0.8 ± 1.3	—2.5 ± 2.5	+3.7 ± 2.5	—963.0[a]	+158.3[d]
	PD/Isc	72.40 ± 13.3	88.24 ± 9.2	169.8 ± 23.7	326.7 ± 44.0	225.7 ± 37.4	166.5 ± 36.0	+92.5[a]	—114.4[a]
Cl^- (5)	PD	+0.5 ± 0.1	+0.2 ± 0.2	+1.3 ± 0.4	+1.4 ± 0.3	0.0 ± 0.3	—0.2 ± 0.5	—725.0[a]	+875.0[a]
	Isc	+13.4 ± 1.7	+1.5 ± 5.7	+15.3 ± 4.0	+17.6 ± 6.3	—1.4 ± 10.6	—6.5 ± 14.3	—902.6[a]	+1,245.7[a]
	PD/Isc	41.5 ± 10.9	46.0 ± 15.4	89.3 ± 20.0	110.7 ± 39.9	78.1 ± 28.0	49.9 ± 10.9	+92.2[a]	—70.1[d]
K^+ (4)	PD	+0.5 + 0.1	+0.2 ± 0.1	+0.4 ± 0.2	+0.1 ± 0.2	+0.5 ± 0.1	+0.3 ± 0.1	—94.4[d]	—250.0[a]
	Isc	+6.8 ± 1.2	+2.7 ± 2.0	+3.5 ± 3.3	+0.7 ± 2.5	+6.3 ± 1.8	+4.5 ± 1.9	—29.3[d]	—256.8[a]
	PD/Isc	78.6 ± 15	105.8 ± 41.4	87.7 ± 21.9	143.3 ± 44	113.7 ± 41.0	133.2 ± 49.8	—17.0[d]	—28.15[d]

Statistical significance (Student's test): a, $p < 0.01$; b, $p < 0.02$; c, $p < 0.05$; d not significant.

Table II. *Electrical potential difference (PD), short-circuit current (Isc) and tissue resistance (PD/Isc) across the duodenum of pigeon. Effects of omission and restitution of ions from only one compartment.*

Experimental condition Ion omitted	Param- eters	Time (minutes) and solutions							
		Standard		Omission		Restitution		Changes %	
		0	24	30	48	54	66	Omission	Restitution
	PD	+0.5± 0.1	—0.3± 0.0	—1.6± 0.4	—3.2± 0.8	—1.6± 0.4	—1.1± 0.3	+364.7[a]	—488.2[a]
Na^+-M	Isc	+11.1± 1.5	—5.2± 1.9	—14.7± 3.2	—21.3± 4.1	—26.6± 8.8	—23.9± 8.6	+184.3[a]	+102.1[d]
(5)	PD/Isc	40.6+ 5.7	97.8±19	135.0±37.4	182.4±51.2	107.2±38.2	84.6±27.7	+38.0[d]	—76.9[c]
	PD	+0.4± 0.2	—0.3± 0.2	+3.8± 0.7	+4.3± 0.5	—2.4± 0.3	—2.4± 0.3	—1,608.0[a]	+2,680.0[a]
Cl^--M	Isc	+7.2± 4.5	—6.4± 3.7	+41.5±10.9	+37.4± 8.9	—29.0±12.2	—32.6±11.9	—746.9[a]	+1,034.4[a]
(6)	PD/Isc	56.4±11.3	83.0±19.3	124.3±28.2	158.3±39.7	131.2±31.9	113.3±26.6	+49.7[b]	—32.7[d]
	PD	+1.0± 0.2	+0.2± 0.1	—0.2± 0.3	—0.1± 0.2	+0.2± 0.1	—0.2± 0.2	+227.8[c]	—200.0[b]
K^+-M	Isc	+21.5± 5.4	+2.0± 0.7	—0.7± 2.5	—0.8± 1.1	+1.3± 0.6	+1.0± 1.7	+133.3[d]	+104.6[b]
(4)	PD/Isc	52.8± 6.9	101.8±20.6	139.3±31.3	174.9±11.5	184.7±51.4	129.4±21.3	+36.9[d]	+9.6[d]
	PD	+1.1± 0.1	—0.1± 0.4	+3.3± 0.3	+2.8± 0.3	+2.2± 0.2	+1.5± 0.1	—2,866.7[a]	+483.3[a]
Na^+-S	Isc	+24.4± 4.3	—2.2± 5.8	+47.6± 6.8	+32.0± 4.7	+26.7± 4.5	+20.3± 3.9	—2,303.2[a]	+247.2[d]
(5)	PD/Isc	47.3± 4.1	63.2± 6.3	83.7±20.1	92.8±14.0	93.6+15.8	83.8±13.0	+32.3[d]	+1.3[d]
	PD	+0.8± 0.2	+0.5± 0.1	—3.9± 0.4	—5.1± 0.3	+2.1± 0.2	+2.1± 0.2	+939.1[a]	—1,582.6[a]
Cl^--S	Isc	+10.9± 1.9	+4.6± 1.2	—43.5±14.8	—47.8±13.2	+17.8± 2.8	+18.3± 3.4	+1,051.6[a]	—1,433.9[a]
(5)	PD/Isc	75 4± 7.6	101.7+15.3	121.2±27.5	132.7±22.9	133.0±22.1	131.4±22.6	+19.2[d]	+0.3[d]
	PD	+0.6± 0.1	+0.3± 0.2	+0.8± 0.1	+0.6± 0.1	+0.5± 0.0	+0.5± 0.0	—127.3[a]	+45.5[b]
K^+-S	Isc	+15.1± 3.6	+5.5± 2.5	+16.2± 3.1	+15.2± 5.8	+9.6± 2.5	+9.1± 2.6	—193.7[a]	+102.4[d]
(4)	PD/Isc	38.1± 3.2	66.0±10.5	52.1±10.9	54.8±12.9	58.5±11.5	58.9±11.5	—21.1[d]	+5.4[d]

Statistical significance, units and conditions as in table I.

Table III. *Electrical potential difference (PD), short-circuit current (Isc) and tissue resistance (PD/Isc) across the ileum of pigeon. Effects of omission and restitution of ions from both compartments.*

Experimental condition Ion omitted	Parameters	Time (minutes) and solutions: Standard 0	Standard 24	Omission 30	Omission 48	Restitution 54	Restitution 66	Changes % Omission	Changes % Restitution
Na^+ (5)	PD	+6.6 ± 0.2	+7.2 ± 0.3	—8.5 ± 0.4	—4.3 ± 0.2	+1.8 ± 0.3	+2.0 ± 0.2	+218.7[a]	—84.7[a]
	Isc	+471.3 ± 44.0	+479.0 ± 38.6	—165.0 ± 23.0	—74.2 ± 14.2	+133.3 ± 21.1	+162.5 ± 21.7	+134.4[a]	—43.3[a]
	PD/Isc	14.4 ± 1.4	15.2 ± 0.2	53.7 ± 6.7	63.6 ± 8.3	14.0 ± 1.0	12.8 ± 0.8	+265.3[a]	—325.4[a]
Cl^- and Ca^{++} (4)	PD	+6.1 ± 0.4	+7.0 ± 0.4	+6.4 ± 0.4	+1.7 ± 0.2	+1.7 ± 0.2	+2.2 ± 0.1	+7.9[d]	—1.2[d]
	Isc	+400.3 ± 15.7	+403.5 ± 28.8	+231.0 ± 8.6	+37.7 ± 5.1	+137.6 ± 25.3	+161.6 ± 17.2	+42.8[d]	—24.8[d]
	PD/Isc	15.2 ± 1.4	17.4 ± 1.5	27.8 ± 2.0	47.9 ± 8.8	13.0 ± 1.3	13.8 ± 1.4	+59.6[d]	—199.6[d]
Cl^- (5)	PD	+6.4 ± 0.2	+7.1 ± 0.3	+6.7 ± 0.2	+1.6 ± 0.5	+1.5 ± 0.2	+1.8 ± 0.1	+5.3[c]	+0.8[d]
	Isc	+374.9 ± 29.8	+454.1 ± 38.2	+243.6 ± 16.0	+49.5 ± 3.8	+105.3 ± 3.7	+122.0 ± 12.9	+46.4[a]	—12.3[a]
	PD/Isc	17.5 ± 1.7	16.3 ± 1.39	27.6 ± 1.1	32.2 ± 2.2	15.0 ± 1.0	15.0 ± 0.8	+68.5[a]	—104.7[a]

Statistical significance, units and conditions as in table I.

Monocompartimental substitution and restoration of ions in duodenum. When the sodium ion was substituted on the mucosal side, both PD and Isc progressed toward serosa negativity with respect to mucosa (table II), but when the substitution took place on the serosal side, results were just the opposite, i.e. the parameters progressed towards positivity (table II). In either case, when the ion was restored to the medium, these parameters tended again towards normality and recovery of their basal values.

Similar effects were produced, although with different polarity, when the chloride was substituted with sulphate. The absence of that ion on the mucosal side provoked an increase in serosa positivity with respect to mucosa both in PD and Isc, whereas such an absence on the serosal side caused the two parameters to tend rapidly towards negativity (table II). Restoring the ion to the medium produced very abrupt changes in the polarity of the parameters, exceeding even the value sobtained under standard conditions.

When the potassium ion was absent from either the serosal or mucosal side the changes observed were similar to those found in the absence of sodium, but of lesser magnitude (table II).

In all these cases, the electrical resistance underwent only slightly significant changes (table II).

Bicompartimental substitution and restoration of ions in ileum. The absence of sodium ions (Tris as substitute) in the incubation medium produced a drop in the transmural potential difference from +7.2 mV to —8.5 mV (serosa negative) on the first measurement taken after the ion substitution. The Isc dropped similarly, reaching —165.0 $\mu A \times cm^{-2}$ on the first measurement taken within the substitution period. When the ion was restored to the medium, both parameters tended towards their basal values, but failed to reach them during the experiment (table III). Tissue resistance underwent an increase just as when sodium was substituted in duodenum.

In the absence of either Cl^- alone or Cl^- and Ca^{++} (table III), identical changes were recorded for PD and Isc, both moving towards zero, although this value was not reached, due probably to the shortness of the substitution period which only lasted 24 minutes. The electrical resistance was somewhat greater when both Cl^- and Ca^{++} were absent than in the absence of Cl^- alone (table III).

Discussion

Ion transport in duodenum. The results obtained in duodenum from pigeon *(Columba livia var. domestica)* show basically an active absorption of sodium (mucosa-serosa), which triggers a passive absorption of chlorine.

The simultaneous absence of the chloride ion from the mucosal and serosal sides produces an increase in both the transmural potential difference and the short circuit current intensity. The increase in serosa positivity, which lasts throughout the ion substitution period, shows that the sodium ion is actively transported from mucosa to serosa. Contrariwise the simultaneous absence of the sodium ion (Tris as substitute) from mucosa and serosa does not alter either the potential difference or the current intensity (table I), which indicates that the chlorine ion is not actively transported, but merely drawn towards serosa by the sodium ion. That would explain the nearly total absence of transmural potential difference and short circuit current intensity under standard conditions.

When the K^+ ion is omitted from the incubation medium on both sides of the membrane, no significant changes are observed in the bioelectric parameters (table I). DIAMOND (7) has reported that K^+

absence from the serosal side in fish gall bladder for a 30 min period, produces a 50 % decrease in the net transport of sodium. In the present study the K^+ ion was absent from the incubation medium for 24 min. If its absence decreased the sodium flux, the effect would not have been detected in the PD and Isc values, since a decrease in sodium transport would have entailed a decrease in the passive flux of the chloride ion, rendering the PD and Isc values unchangeable. The great increase in tissue resistance from the bicompartimental substitution of sodium constitutes another important change (table I). ANDO *et al.* (1) have observed a continuous increase in tissue resistance in the anterior intestine of eel when sodium is absent from the incubation medium. Identical result has also been reported by BADÍA and LORENZO (2) in fish *Gobius maderensis*.

When ions are omitted only from either side, tissue permeability can be readily observed. When the same ion is absent from either the mucosa or the serosa, the results are the same in magnitude but of opposite sign (table II). Consequently the ion present only in one side of the tissue seems to undergo passive diffusion with the greatest of ease towards the other side in order to balance its concentration in the two compartments. This effect takes place in the substitutions of either sodium, chlorine or potassium ion, although the variations in the last case are much smaller and probably due to its low concentration in the extracellular liquids.

Ion transport in ileum. When ileum was incubated in KRT-glucose, the standard solution, the values for PD and Isc were 7.2 mV (serosa positive) and 444 $\mu A \times cm^{-2}$ respectively. Similar results have been found by SCHULTZ and ZALUSKY (14) in rabbit ileum.

When the sodium ion is substituted by Tris on both ileum sides, PD reverses from positive to negative values (table III), which brongs out clearly the passing of chloride ions towards serosa. These results agree with those obtained by VÁZQUEZ *et al.*, who *measured* PD in the absence of sodium in the small intestine of hen (18).

A decrease in both PD and Isc towards zero values (table III) occurred when the Ca^{++} and Cl^- ions were absent simultaneously or just in the absence of the Cl^- ion, as opposed to what happened in duodenum, where the substitution of the chloride ion increased the serosa positivity. It is deduced from the above that the lack of choline in the incubation medium in ileum inhibits the sodium transport, otherwise observed in the control medium, which might indicate the existence of a possible coupling between the sodium and the chlorine transports, the proof of which would require the estimation of ionic fluwes. NEALLANS and FRIZELL (13) have proven the existence of a coupled transport for Na^+ and Cl^- in ileum of rabbit.

Resumen

Se estudia en duodeno e íleon de paloma *(Columba livia var. domestica)*, en soluciones de diferente composición iónica, la diferencia de potencial transmural (DP), intensidad de corriente de cortocircuito (Isc) y resistencia tisular.

Cuando el tejido es incubado en una solución estándar, la DP a través del duodeno es aproximadamente cero, mientras que a través del íleon es de 7,2 mv (serosa positiva respecto a mucosa).

Las medidas de la DP e Isc en el duodeno, en ausencia de sodio por una parte y de cloro por otra, ponen de manifiesto una absorción activa de sodio desde mucosa a serosa, la cual arrastra cloro en el mismo sentido.

Cuando el sodio es sustituido en el íleon (Tris como sustituto), la DP e Isc muestran un marcado aumento de la negatividad de la serosa. En ausencia de Cl^- (sulfato como sustituto) en íleon, la DP e Isc tienden a cero, lo

que indica que la presencia del ion cloruro es necesaria para el transporte de sodio.

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