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Asymmetrical oxygen availability from serosal and luminal sides of rat distal colon epithelium

F. D. Saraví, T. A. Saldeña, L. M. Cincunegui and G. E. Carra

Cátedra de Física Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza 5500 (Argentina)

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Short-circuit current (Isc) and transepithelial potential difference (PD) of the rat distal colon mucosa are sensitive to acute hypoxia *in vitro*. The relative contribution of luminal and serosal oxygenation in sustaining Isc and PD was assessed. Rat distal colon Isc and PD responses to hypoxia and reoxygenation of preparations of mucosa-submucosa, and of isolated mucosa (with and without the mucus gel layer), mounted in an Ussing chamber, and of sacs of everted and non-everted isolated mucosa, were measured. In Ussing chambers, a 5-min total (bilateral) hypoxia reduces Isc and PD by 50 to 70 %, while an overshoot was observed on reoxygenation. Serosal hypoxia caused about the same effect as total hypoxia, with complete recovery on reoxygenation. Luminal hypoxia had no effect in either Isc or PD. After total hypoxia, selective serosal reoxygenation allowed complete recovery of Isc and PD; addition of luminal reoxygenation did not further increase Isc and PD. Luminal reoxygenation after total hypoxia did not modify the decrease in Isc and PD, but addition of serosal reoxygenation led to complete recovery. A similar behaviour was seen in isolated mucosa preparations without the mucus gel layer. Baseline Isc and PD of everted sacs were about 45 % of those of non-everted sacs, but their response to a hypoxic challenge was slightly attenuated. On reoxygenation, both everted and non-everted sacs showed complete recovery. Summing up: serosal oxygenation is both necessary and sufficient to sustain rat distal colon Isc and PD, while luminal oxygenation is not; there seems to exist a barrier, different from the mucus gel layer, for oxygen access from the luminal side of the epithelium; and distal colon isolated mucosa everted sac preparations are suboptimally oxygenated.

Key words: Epithelial polarity, Hypoxia, Intestinal sacs, Rat distal colon, Isc, Ussing chamber.

Correspondence to F. D. Saraví (Phone: 54 61 20 5020, Ext. 2646; Fax: 54 61 38 0232).

Intestinal ion transport is coupled to oxidative metabolism. For example, in rabbit distal colon *in vitro* it has been estimated that 15 to 20 sodium ions are transported per oxygen molecule consumed (7). *In vivo*, oxygen diffuses from the capillary network surrounding the colonic crypts (9) and reaches the epithelial cells via their basolateral membranes. Under in vitro conditions, such as those found in an Ussing chamber, oxygen is usually supplied to both the luminal and the serosal sides.

Acute hypoxia induced by switching the gassing of both sides of an Ussing chamber from 95 % O_2 - 5 % CO_2 to 95 % N_2 - 5 % CO_2 causes fast decreases in short-circuit current (Isc) and transepithelial potential difference (PD). Both Isc and PD overshoot above baseline values upon reoxygenation after a 5 min-hypoxic period, and show complete recovery after up to 15-min, but not after 20-min hypoxic periods (23).

The present study was carried out to determine if the effect of inducing hypoxia on the luminal side differs from that of inducing it on the serosal side, and whether the effects of serosal and luminal hypoxia are additive or synergic. A markedly asymmetric electrical response was found, serosal hypoxia having a far more intense effect than luminal hypoxia. After total (bilateral) hypoxia, serosal reoxygenation was able to restore Isc and PD, while luminal reoxygenation was not. Mechanical removal of the mucus gel layer had no significant effect in the response to luminal reoxygenation after total hypoxia. Furthermore, while preserving their response to hypoxia and reoxygenation, everted sacs showed lower baseline Isc and PD than non-everted sacs, suggesting that the former are suboptimally oxygenated.

Materials and Methods

Male adult Wistar rats (250-300 g BW) fed on a standard diet (Carhill Co.) and tap water ad libitum, were used. Animals were housed and managed according to the guidelines for animal care and biosafety of the Medical School.

Dissection and Mounting

Ussing chamber experiments.– Tissues were prepared as previously described (23). Under ether anesthesia, the entire colon was excised and rinsed free of contents with cold (4 °C) Ringer solution. A 5-mm diameter plastic rod was inserted into a 2.5-cm segment cut from the descending colon just above the pelvic brim. Dissection was performed in cold oxygenated Ringer solution under a stereomicroscope. With both fine scissors and forceps the segment was either stripped free of serosa and muscular layers to obtain a mucosa-submucosa preparation, or of serosa, muscular and submucosal layers to obtain an isolated mucosa preparation. It was then cut open along its mesenteric border and mounted as a flat sheet in an Ussing-type chamber (opening = 1 cm^2), kept at 37 ± 0.5 °C and allowed to stabilize for 70 to 90 min. In another series of experiments, after extending isolated mucosa as a flat sheet, and before mounting it in the chamber, surface mucus gel layer was carefully removed with a cotton tip soaked in Ringer solution.

Intestinal sacs.– Three to 4-cm segments were dissected to obtain isolated mucosa preparations. To minimize epithelial edge damage effects, cuffs of whole wall were left at both ends, where the sac was tied to the holding apparatus with or without previous eversion. At the end of the electrical measurements, meth-

ylene blue was instilled into the sac to detect leaks. Sac preparations were oxygenated by bubbling 95 % O_2 - 5 % CO_2 in the external solution through a porous glass diaphragm located at the base of the 100-ml outer compartment.

Electrical Measurements

PD was recorded with calomel electrodes connected to the chamber or the sac reservoirs through 3 % Agar-in-Ringer bridges. An amplifier allowed current to be passed through Ag-AgCl electrodes, clamping the PD at 0 mV with correction for bridge asymmetry and compensation for solution resistance. For experiments with sacs, the inner current electrode was straight, and the outer electrode had an helicoidal shape. Isc was monitored with a digital display and a chart recorder. The clamp was released every 5 min, at the end of each hypoxic period, and on reoxygenation, to allow measurement of open-circuit PD. Tissue resistivity (Rt) was calculated from Isc and PD values after Ohm's law. The surface of sacs was measured at the end of the experiment to calculate Isc in $\mu A \cdot cm^{-2}$ and Rt in $\Omega \cdot cm^2$.

Solution and Gases

The Ringer solution had the following composition (mmol/L): 132.8 Na^+; 114.0 Cl^-; 4.5 K^+; 24.0 HCO_3^-; 0.8 HPO_4^{2-}; 0.2 $H_2PO_4^-$; 1.25 Ca^{2+}; 1.0 Mg^{2+}; 1.0 SO_4^{2-}; 10.0 D(+)glucose. The solution was gassed with either humidified 95 % O_2 - 5 % CO_2 during control conditions and on reoxygenation, or with 95 % N_2 - 5 % CO_2 during hypoxia. Solution pH was 7.40 when gassed with either gas mixture.

Experimental Procedures

In intestinal sacs, a 5-min hypoxic period was followed by reoxygenation. In experiments with Ussing chambers, one of the two following procedures was carried out.

Total and selective hypoxia and reoxygenation.- In mucosa-submucosa and isolated mucosa preparations, in random order: 5-min total (bilateral) hypoxia, followed by reoxygenation; 5-min luminal hypoxia, followed by luminal reoxygenation; 5-min serosal hypoxia, followed by serosal reoxygenation. Between two successive hypoxic challenges, a 30-min period was allowed for equilibration.

Total hypoxia with sequential reoxygenation.– In isolated mucosa preparations, in random order: 5-min total (bilateral) hypoxia followed by serosal reoxygenation, and after an additional 5-min period by luminal reoxygenation (*Sequence 1*) and 5-min total hypoxia, followed by luminal reoxygenation, and after an additional 5-min period, by serosal reoxygenation (*Sequence 2*).

Microscopy

The efficacy of mucus gel layer mechanical removal was assessed by visualization of thick cuts (1 mm) of freshly dissected tissue by direct light microscopy, with an adaptation of methods employed for measuring gastric gel mucus layer thickness (14, 21, 22). Integrity of preparations, in which the mucus gel layer was removed, was assessed by light microscopy with standard hematoxilin-eosin staining.

Statistics.– Comparison of three or more groups was performed by Bonferroni's method. For comparison of two means, a two-sided Student's *t* test for unpaired data was employed. Values are

reported as means ± SEM. Values of $p < 0.05$ were considered significant.

Results

Total and selective hypoxia and reoxygenation

Isc, PD and Rt values for total, serosal and luminal hypoxia and reoxygenation for mucosa-submucosa and isolated mucosa preparations are shown in table I. For both types of preparation, responses to total and serosal hypoxia and reoxygenation were similar. Luminal hypoxia caused a slight depression of Isc and PD in mucosa-submucosa preparations, but no change at all in isolated mucosa preparations; neither Isc nor PD overshooted on reoxygenation.

Total hypoxia followed by sequential oxygenation

In *sequence 1*, serosal reoxygenation was usually followed by an Isc peak that afterwards plateaued at a value very close to baseline, which did not change by the addition of luminal reoxygenation. In *sequence 2*, luminal reoxygenation was unable to restore baseline Isc and PD, but subsequent serosal reoxygenation was (table II).

Effect of mechanical removal of the mucus gel layer

Careful mechanical cleaning removed the mucus gel layer, without affecting epithelial surface integrity, as assessed by light microscopy. These preparations reached steady baseline values undistinguishable from preparations without removal of the mucus gel layer. In total versus selective hypoxia (table I), the response to serosal hypoxia remained similar to the response to total hypoxia, while luminal hypoxia showed no effect. No change in the response to *sequence 1* was detected. However, in *sequence 2* a non-significant trend towards a response to luminal reoxygenation was noticed (table II).

Experiments with intestinal sacs

Baseline Isc, PD and Rt for everted sacs ($n = 8$) were, respectively, 66.4 ± 5.2 $\mu A \cdot cm^{-2}$, 2.6 ± 0.1 mV and 40.8 ± 3.9 $\Omega \cdot cm^2$; corresponding values for non-everted sacs ($n = 8$) were 150.8 ± 23.7 $\mu A \cdot cm^{-2}$, 6.6 ± 1.0 mV and 47.8 ± 6.3 $\Omega \cdot cm^2$. The differences were significant for both Isc ($p < 0.01$) and PD ($p < 0.01$), but not for Rt. Non-everted sac preparations lasted longer (6 to 8 h) than everted sacs (3 to 4 h). The Isc response of non-everted sacs to hypoxia was significantly less intense than that of non-everted sacs, when expressed either in $\mu A \cdot cm^{-2}$ ($p < 0.01$) or as a percentage of baseline values ($p < 0.05$). On reoxygenation, both types of preparation showed complete recovery (fig. 1). No significant change of Rt was seen during hypoxia and reoxygenation in either preparation.

Discussion

Colonic gas composition is variable, differs sharply from that of inspired air and its pO_2 does not match those of either arterial or venous blood (16, 28). Explanations advanced for the low colonic oxygen concentration include shunting of blood oxygen by a countercurrent exchange, consumption by the bacterial flora, and consumption by the mucosa itself. The contribution of the first factor seems to be negligible (13). Consumption by colonic bacteria may contribute to lower oxygen concentration; still, the mean colonic

Table I. *Responses to total and selective hypoxia and reoxygenation of rat distal colon epithelium in an Ussing chamber*
Preparations of mucosa-submucosa (n = 11), isolated mucosa (n = 12) and isolated mucosa with mechanical removal of the mucus gel layer (clean isolated mucosa; n = 6) were submitted to a 5-min total (bilateral) hypoxia followed by reoxygenation from both sides of the chamber, or to a 5-min hypoxia from either the serosal or luminal side followed by reoxygenation (while maintaining oxygenation to the opposite side throughout). Tabulated figures (mean ± SEM) were taken at the end of the hypoxic period and at the moment of maximal Isc reached on reoxygenation. An asterisk indicates a significant difference from the respective baseline.

	Total			Serosal			Luminal		
	Isc $\mu A \cdot cm^{-2}$	PD mV	Rt $\Omega \cdot cm^2$	Isc $\mu A \cdot cm^{-2}$	PD mV	Rt $\Omega \cdot cm^2$	Isc $\mu A \cdot cm^{-2}$	PD mV	Rt $\Omega \cdot cm^2$
Mucosa-submucosa									
Baseline	64.4 ± 3.9	8.0 ± 0.7	124.8 ± 11.4	65.6 ± 8.2	7.0 ± 1.1	105.3 ± 8.2	67.4 ± 7.4	6.9 ± 0.9	103.9 ± 10.2
Hypoxia	21.3 ± 3.7*	2.7 ± 0.4*	132.8 ± 13.5	18.5 ± 3.5*	2.1 ± 0.4*	109.9 ± 9.1	57.5 ± 6.2	5.7 ± 0.8	98.0 ± 9.8
Reoxygenation	84.4 ± 4.5*	9.4 ± 0.6	114.1 ± 9.5	80.9 ± 8.3	7.8 ± 1.0	95.4 ± 9.0	55.9 ± 4.7	5.4 ± 0.7	95.6 ± 8.5
Isolated mucosa									
Baseline	83.2 ± 8.8	8.8 ± 0.8	112.2 ± 9.5	90.1 ± 7.6	9.9 ± 0.8	113.7 ± 9.4	84.9 ± 8.7	9.4 ± 1.1	113.5 ± 8.9
Hypoxia	36.7 ± 6.2*	4.0 ± 0.7*	114.2 ± 10.1	47.3 ± 5.5*	5.6 ± 0.8*	118.2 ± 11.1	88.8 ± 8.7	9.5 ± 1.1	109.2 ± 8.3
Reoxygenation	118.1 ± 8.6*	12.1 ± 0.9*	106.0 ± 8.6	121.0 ± 9.2*	13.2 ± 1.4	108.2 ± 8.9	93.3 ± 8.7	9.8 ± 1.1	108.4 ± 8.7
Clean isolated mucosa									
Baseline	103.2 ± 6.9	11.3 ± 0.8	109.5 ± 2.9	99.3 ± 8.6	9.8 ± 1.0	98.0 ± 3.3	95.5 ± 7.0	9.8 ± 1.0	102.2 ± 4
Hypoxia	55.3 ± 6.7*	5.7 ± 0.8*	103.7 ± 3.6	54.7 ± 8.1*	5.4 ± 0.8*	98.2 ± 3.1	96.5 ± 8.3	9.4 ± 0.9	97.0 ± 3.9
Reoxygenation	117.0 ± 8.6	11.9 ± 1.0	102.5 ± 4.2	106.7 ± 9.9	10.4 ± 0.9	97.5 ± 2.9	94.5 ± 8.3	9.1 ± 0.9	96.5 ± 3.7

Table II. *Effect of total hypoxia and sequential reoxygenation of rat distal colon epithelium in Ussing chamber.*

Preparations of isolated mucosa (n = 16) and isolated mucosa with mechanical removal of the mucus gel layer (clean isolated mucosa, (n = 7) were submitted to 5-min total hypoxia followed by sequential serosal-luminal (Sequence 1) or luminal-serosal (Sequence 2) reoxygenation. Figures (mean ± SEM) are those reached at the end of the hypoxic period and 5 min after luminal or serosal reoxygenation. An asterisk indicates a difference from the respective baseline.

	Isolated mucosa			Clean isolated mucosa		
	Isc $\mu A \cdot cm^{-2}$	PD mV	Rt $\Omega \cdot cm^2$	Isc $\mu A \cdot cm^{-2}$	PD mV	Rt $\Omega \cdot cm^2$
Sequence 1						
Baseline	98.2 ± 6.9	9.8 ± 0.7	102.2 ± 5.5	86.0 ± 6.0	9.4 ± 1.1	116.2 ± 12.4
Hypoxia	40.8 ± 4.7*	4.3 ± 0.6*	104.1 ± 6.4	47.2 ± 7.4*	4.9 ± 0.6*	114.2 ± 10.5
Serosal Reox.	92.8 ± 5.9	9.2 ± 0.7	100.0 ± 5.2	100.2 ± 8.7	10.1 ± 1.5	108.0 ± 13.9
Luminal Reox.	93.7 ± 5.3	9.1 ± 0.6	98.4 ± 5.3	94.0 ± 7.4	9.8 ± 1.5	109.8 ± 12.6
Sequence 2						
Baseline	108.9 ± 6.4	11.7 ± 0.9	106.8 ± 5.0	97.7 ± 5.6	9.9 ± 1.6	106.4 ± 11.1
Hypoxia	59.0 ± 6.3*	6.3 ± 0.8*	104.7 ± 5.3	40.5 ± 8.9*	4.0 ± 1.1*	109.6 ± 10.3
Luminal Reox.	53.2 ± 5.3*	5.8 ± 0.6*	109.8 ± 5.6	56.5 ± 8.9*	5.7 ± 1.6*	112.4 ± 10.4
Serosal Reox.	117.7 ± 9.5	11.8 ± 1.1	97.7 ± 4.5	103.5 ± 11.1	10.2 ± 1.9	103.8 ± 10.1

mucosa pO_2 of germ-free rats is 15 mmHg (4). Epithelial oxygen consumption may be more important (13). However, the results here reported suggest yet another factor, namely that the mucosa itself acts as a limiting barrier for oxygen diffusion.

Previous reports of the effect of hypoxia on colon Isc in Ussing chambers have employed total (bilateral) hypoxia (8, 17, 23). Present data show that rat distal colon epithelium reacts to serosal hypoxia and reoxygenation essentially in the same way as to total hypoxia, both in mucosa-submucosa and in isolated mucosa preparations. Luminal hypoxia had no noticeable effect in either preparation. On the other hand, in the isolated mucosa, serosal reoxygenation after total hypoxia led to complete Isc and PD recovery, while luminal reoxygenation did not. This is in line with the observation that in the isolated rat colon perfused *in vitro* through the vascular bed, oxygenation of the intraluminal perfusion solution has but a small effect in oxygen extraction from the vascular perfusate (19). The asymmetry may be peculiar to the colon, since the small intestine may be adequately oxygenated from the luminal side both *in vitro* (2, 18) and *in vivo* (1, 12, 24).

Since the adherent mucus gel layer is a diffusive barrier for ions and molecules (3), it could have a role in causing the observed asymmetry. One way to assess the role of the mucus gel layer would be to use a mucolytic agent. Although results obtained with one such drug, dithiothreitol (1 mmol/L) suggests that the mucus gel layer is not primarily responsible for the asymmetry, the agent itself was found to cause a clear reduction of Isc and PD (21). For this reason, mechanical removal of the mucus gel layer was employed in the present work. The procedure reliably

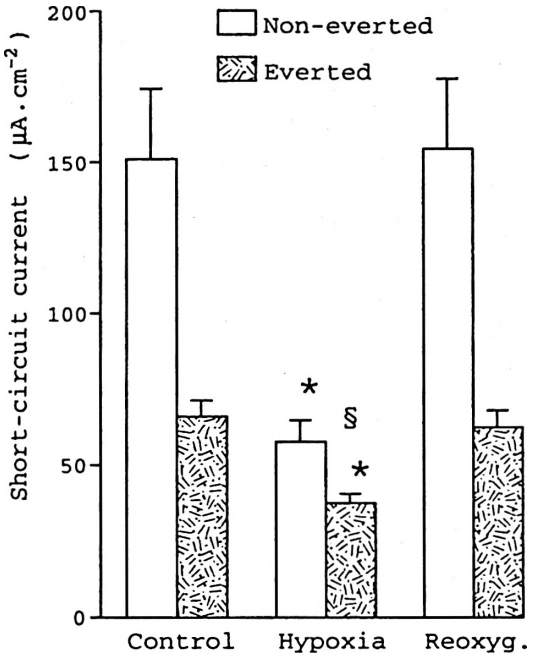

Fig. 1. *Response of sacs of rat distal colon isolated mucosa to hypoxia and reoxygenation.*
Short-circuit current responses of sacs of non-everted (n = 8) and everted (n = 8) rat distal colon isolated mucosa submitted to a 5-min hypoxic challenge followed by reoxygenation; (*) indicates a significant difference from the respective control value, and (§) a significant difference in the response to hypoxia of everted versus non-everted sacs. No difference from baseline was observed on reoxygenation in either type of preparation.

resulted in structurally and functionally adequate isolated mucosa preparations devoid of a superficial mucus gel layer. Mucus secretion continued during the experimental period, but the structured, adherent mucus gel layer was not restored. The only caveat is that mechanical cleaning removes the surface layer, but not crypt mucus.

The response to total, serosal and luminal hypoxia and reoxygenation of mechanically cleaned preparations was similar to that of non-cleaned preparations, except for the lack of a significant overshoot of Isc and PD on total and serosal reoxygenation. In sequential reoxygenation after total hypoxia, preparations without the mucus gel layer behaved like normal isolated mucosa with the *sequence 1* (serosal reoxygenation first). On the other hand, in the *sequence 2* they showed a non-significant trend towards recovery of about 15 % in Isc and PD with luminal reoxygenation. Still, as recovery did not reach significance, it may be concluded that the mucus gel layer is a minor contributor to the observed asymmetry.

The everted sac preparation was introduced for the study of small intestine transport (27) but it has been employed for large intestine as well (5, 11). However, lower baseline Isc and PD values were found for the everted (oxygenated from the luminal side) than for non-everted rat distal colon isolated mucosa sacs. The differences are not attributable to inadvertent damage caused by the eversion procedure, since 1) the degree of stretching during eversion was not greater than the stretching caused by filling the sac with Ringer solution; 2) there was no significant difference between the Rt of everted and non-everted sacs and 3) no leaks were detected when methylene blue was instilled into the sacs at the end of each experiment. These results suggest that everted sac oxygenation is less than optimal, and are consistent with the other observations indicating a predominant role of serosal over luminal oxygen supply for sustaining baseline Isc and PD. Thus, the everted sac preparation may be unsuitable for rat distal colon physiological and pharmacological studies.

No qualitative difference in the response to hypoxia and reoxygenation of everted and non-everted sacs was noticed,

but the Isc and PD hypoxic depression was smaller in the latter. The smaller Isc and PD depression of non-everted sacs with a deliberate hypoxic challenge may be related to the fact that these preparations are already in a state of relative hypoxia.

The results here reported may be explained by different oxygen permeability properties of apical and basolateral membranes. While definitive proof of this hypothesis would demand intracellular PO_2 measurements, work from other laboratories has shown low apical permeabilities to highly lipophilic molecules like ammonia and carbon dioxide in several epithelia (6, 10, 15, 26), including isolated, perfused single colonic crypts (25), and the crypt-like human intestinal cell line T84 (20).

In summary, the evidence presented points to a marked asymmetry in oxygen availability from the basolateral and apical membranes of rat distal colon epithelium, which is not explained by the mucus gel layer. Elucidation of the exact nature of the permeability barrier must wait further work.

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F. D. SARAVÍ, T. A. SALDEÑA, L. M. CINCUNEGUI y G. E. CARRA. *Asimetría en la disponibilidad de O_2 del epitelio de colon de rata desde mucosa o serosa.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (4), 367-376, 1997.

La corriente de cortocircuito (Isc) y la diferencia de potencial transepitelial (PD) del colon distal de rata son sensibles a la hipoxia aguda *in vitro*. Se evalúa la contribución relativa de la oxigenación luminal y serosal para mantener la Isc y la PD y se estudian sus respuestas en preparaciones de mucosa-submucosa y de mucosa aislada (con la capa de gel de mucus y sin ella), montadas en una cámara de Ussing, y la de sacos de mucosa aislada. En cámara de Ussing, una hipoxia total (bilateral) de 5 min de duración inhibe la Isc y el PD en 50 a 70 %; en la reoxigenación se observa un rebote de ambas variables. La hipoxia serosa produce un efecto similar a la hipoxia total, con recuperación completa en la reoxigenación. La hipoxia luminal no tiene efecto sobre la Isc ni sobre el PD. Después de hipoxia total, la reoxigenación selectiva de la serosa permite una recuperación completa de ambos, sin que la adición de reoxigenación luminal produzca aumento adicional. La reoxigenación luminal después de hipoxia total no modifica la reducción de Isc y PD, pero el agregado de reoxigenación serosal lleva a la recuperación completa. Se observa un comportamiento similar en las preparaciones con limpieza mecánica del mucus. Los valores basales de Isc y PD de sacos evertidos son de un 45 % respecto de los no evertidos, aunque su respuesta a la hipoxia resulta algo atenuada. En la reoxigenación, ambos tipos de sacos muestran recuperación completa. Se concluye que, en el colon distal de rata, la oxigenación del lado seroso es necesaria y suficiente para mantener las Isc y PD basales, no siéndolo la oxigenación luminal; que parece existir una barrera, diferente de la capa de gel de mucus, al acceso de oxígeno a las células desde el lado luminal del epitelio; y que las preparaciones de sacos evertidos de mucosa aislada están subóptimamente oxigenadas.

Palabras clave: Cámara de Ussing, Colon distal, Isc, Hipoxia, Polaridad epitelial, Sacos intestinales.

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