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Research Article

# Intestinal drug absorption kinetics determined in Ussing chambers is affected by the nature of the physiological media

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## Abstract

The aim of this study was to determine whether the nature of the physiological medium influences experimentally determined jejunal permeability. Acetaminophen (paracetamol) and vitamin C were used because their absorption pathways are well characterized. The study showed that the composition of the physiological solutions influenced (i) the conductance (G) of the intestinal mucosa and (ii) the intestinal permeation of acetaminophen and vitamin C. After 2 h, the transintestinal flux (Jms) of vitamin C was J=31.4 ± 1.44 $\mu g.h^{-1}.cm^{-2}$ with Ringer's solution, while with Sorensen's solution it was J=51.1 ± 7.09 $\mu g.h^{-1}.cm^{-2}$ and J=15.8 ± 1.92 $\mu g.h^{-1}.cm^{-2}$ with sodium-free Ringer's solution. The acetaminophen flux was J=17.9 ± 1.39 $\mu g.h^{-1}.cm^{-2}$ with Ringer's solution, while with Sorensen's solution it was J=12.6 ± 1.99 $\mu g.h^{-1}.cm^{-2}$. A strong correlation ($R^2$ = 0.979) was observed between conductance and vitamin C fluxes with different solutions, including Ringer's, Sorensen's, and sodium-free Ringer's. For acetaminophen, the correlation was significant only with Ringer's solution ($R^2$ = 0.976). The composition of the physiological solutions also influenced tissue responses to pharmacological effectors such as glucose and carbacholine. The proximal jejunum, when incubated in Ussing chambers, exhibited less morphological deterioration of epithelial villi. In conclusion, this study demonstrates that commonly used physiological media can affect the functional viability of intestinal tissue and influence experimental permeability data, highlighting the importance of carefully considering medium composition in in vitro intestinal drug absorption studies.

**Keywords**: Intestinal absorption, physiological buffers composition, rat intestine, jejunum, physiological media, Ussing chambers, vitamin C, acetaminophen.

## 1. INTRODUCTION

After oral administration of solid dosage forms, drug bioavailability depends on many physicochemical characteristics, including aqueous solubility, ionization, potential adsorption to mucus glycoproteins, variations in dissolved drug concentrations along the gastrointestinal tract, and the resistance of the intestinal mucosal membrane to drug passage from the luminal to the serosal side. This resistance is often described by the apparent permeability coefficient of the drug. Although the primary barrier to diffusion is formed by the tightly connected epithelial cells, the mucus gel layer coating the luminal surface provides an additional barrier. Recent studies have shownto consider that this mucus layer forms an unstirred aqueous layer that can significantly limit intestinal permeability [1,2 3 4] . *In vitro*, the experimental conditions and tissue environment are important factors to consider when evaluating intestinal drug permeability. Transport of drugs, nutrients, xenobiotics, and electrolytes may differ across intestinal regions such as the jejunum, ileum, and colon [5 6].

From an experimental standpoint, intestinal permeability can be assessed in humans and animals using various models: cell culture monolayers, intestinal tissues mounted in Ussing chambers, the everted gut sac technique, or in vivo intestinal perfusion. Among these methods, Ussing chambers allow the study of molecule transport across intestinal epithelium under realistic and controlled in vitro conditions.

They also enable continuous monitoring of electrical parameters that reflect membrane characteristics and functional viability.

Because many drugs require specific formulations due to low solubility, instability, or other limitations, the Ussing chamber model provides a valuable tool to study how such formulations affect intestinal transport. This approach requires confirming the robustness of the

model under conditions that may slightly differ from classical physiological settings.

In the Ussing chamber, the biological preparation (tissue or epithelial monolayer) is mounted between two half chambers that define a mucosal compartment and a serosal compartment. Both sides must be maintained at constant temperature, hydrostatic pressure, and ionic composition to simulate physiological conditions.

Experimentally, it is therefore relevant to investigate how the composition of physiological buffers commonly used for permeability studies affects tissue function and electrical resistance during experiments.

In complex media, certain components may induce cellular toxicity, resulting in data inaccuracies and misinterpretation. These physiological solutions may include salts and additives (e.g., albumin, ethanol), which can directly or indirectly influence intestinal permeability either by interacting with the tested molecule (e.g., instability) or by exerting toxic effects on the epithelium. Consequently, the choice of physiological solutions is critical. For example, permeability studies with Caco2 monolayers are often conducted using culture media such as Dulbeccos Modified Eagle Medium (DMEM), whereas freshly dissected intestinal segments are frequently bathed in Ringer, Tyrode, phosphate buffered saline, or Sorensen solutions in Ussing chamber experiments.

The objective of the present study was therefore to determine whether the intestinal permeability of drugs in vitro is affected by the composition of the experimental solutions. Two high permeability model compounds vitamin C and acetaminophen were selected. Ussing chambers were used to quantify their transport rates. Vitamin C (VC) is actively transported at low concentrations via sodium dependent transporters SVCT1 and SVCT [6 7] and passively transported at higher concentrations. Acetaminophen is mainly absorbed by passive diffusion [8], although interactions with P glycoprotein have been reported [9].

Histology, unidirectional flux measurements, and transepithelial electrical conductance (Gt) were used to compare the effects of different physiological media on tissue viability and drug transport across the intestinal barrier.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

All reagents were purchased and used exclusively for research purposes. Carbacholine hydrochloride, D glucose, D mannitol, vitamin C (L ascorbic acid), paracetamol (acetaminophen), hydroxypropyl β cyclodextrin (HPβCD), and L glutamine (all from Sigma, St. Quentin Fallavier, France) were of analytical grade. DMEM (Dulbecco's Modified Eagle Medium) was obtained from Invitrogen (Saint Aubin, France).

### 2.2. Animals

Mature male Sprague Dawley rats (180–250 g) were obtained from Janvier SAS (Le Genest Saint Isle, France). Animals were housed individually and fed standard laboratory chow (UAR, Villemoisson sur Orge, France).

The study complied with the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and every effort was made to minimize animal suffering and to reduce the number of animals used. Ethical approval was obtained from Paris Diderot University Paris 7, conformed Directive 2010/63/EU of the European Parliament and of the council [10].

Animals were fasted for 18 hours prior to the experiments, with free access to water. They were sacrificed by $CO_2$ inhalation, and the jejunum was removed and rinsed with ice cold Ringer's solution to eliminate intestinal contents. The tissue was opened along the mesenteric border, and fragments were mounted as flat sheets between the two halves of methacrylic Ussing chambers, as previously described [4 11 12]

### 2.3. Deliberate alteration of the tissue with KCl 2%

Altered jejunum fragments were used as controls following the procedure of Myers et al. [13]. Briefly, Ringers solution containing 2% KCl was introduced into the serosal side of jejunal loops for 30 min to induce controlled tissue damage. After incubation, the loops were rinsed with ice cold water (corresponding to the solution used in the experiment), and the tissue was mounted as flat sheets in Ussing chambers.

### 2.4. Transepithelial electrical conductance

Different physiological media (Table 1) were used throughout the experiments. These solutions filled the mucosal (luminal) and serosal (blood side) reservoirs of the Ussing chambers, separated by the jejunal mucosa. In some experiments, the two compartments contained different media.

The pH of the media ranged from 7.0 to 7.40 at 37°C when aerated with a 95% $O_2$ / 5% $CO_2$ mixture.

The spontaneous transmural potential difference (PD) was measured using 3 M KCl/agar bridges connected to calomel electrodes and a high impedance voltmeter. PD was then short circuited and maintained at 0 mV by applying a short circuit current (Isc) using stainless steel electrodes and an automatic voltage clamp system (JFD 1V, Laboratoires TBC & Biomécatronics SAS, France).

Delivered $I_{sc}$, corrected for fluid resistance, was recorded continuously using Biodaqsoft software (Laboratoires TBC &Biomécatronics SAS, Ruitz, France).

The $I_{sc}$ (in μA/cm²) represents the net transepithelial ion flux (primarily $Na^+$, $Cl^-$, and $HCO_3^-$) in the absence of an electrochemical gradient (mainly $Na^+$, $Cl^-$, and $HCO_3^-$). Transepithelial electrical conductance (Gt) was calculated using Ohm's law and expressed as mS/cm².

At the end of the experiments, tissue viability was assessed by adding glucose or carbacholine ($10^{-4}$ M), and recording their characteristic electrical responses.

**Table1: Physiological media composition**

| Code number | Media | Composition | Osmolarity (mOsm) | pH |
|---|---|---|---|---|
| M1 | Ringer | 115 mMNaCl; 25 mM $NaHCOO_3$; 1,2 mM $CaCl_2$, $2H_2O$, 1,2 mM $MgCl_2$, $H_2O$; 2,4 mM $K_2HPO_4$; 0,4 mM $KH_2PO_4$ | 278 | 7.4 |
| M2 | $Na^+$-free-Ringer | 5,7mM choline chloride); 1,25mM $KHCO_3$ 1,2mM $CaCl_2$,$2H_2O$, 1,2mM $MgCl_2$, $2H_2O$ ; 2,4mM $K_2HPO_4$; 0,4mM $KH_2PO_4$ | 168 | 7.4 |
| M3 | Phosphate Buffer (Sörensen's buffer) 0,1M | 22mM $NaH_2PO_4$; 101mM $Na_2HPO_4$ | 200 | 7.4 |
| M4 | PBS (phosphate buffered saline) | 137 mM NaCl; 2,7 mMKCl, 10 mM $Na_2HPO_4$, 1,76 mM $K_2HPO_4$ | 315 | 7.4 |
| M5 | Reduce Oral rehydratation solution (SRO-r) | 44 mMNaCl; 20 mMKCl; 78 mM glucose anhydride; 79 mM(citrate trisodique) | 245 | 7.0 |
| M6 | Standard oral rehydratation solution (SRO-s) | 44 mMNaCl; 20 mMKCl; 111mM glucose anhydride; 79 mM (citrate trisodique) | 311 | 7.4 |
| M7 | DMEM (Dulbecco's Modified Eagle Medium) | Complex composition, including glucose; sodium pyruvate; a cocktail of amino acids , glutaMAX™; phenol Red | 320 | 7.2 |
| M8 | NaCl 0,9% | 154 mMNaCl | 308 | 7.4 |

### 2.5. Vitamin C and acetaminophen transepithelial fluxes

Once electrical parameters reached steady state, tissues were paired according to their conductance values (± 20%). When $I_{sc}$ stabilized, vitamin C (1 mg/mL) and acetaminophen (AP, 1 mg/mL) solutions were added to the mucosal compartment. Samples (1 mL) were collected from the serosal compartment at 0, 30, 60, 90, and 120 min and replaced with fresh medium at 37°C. VC and AP samples were then diluted to 3 mL with ultrapure water and analyzed by UV spectrophotometry at 286 nm (vitamin C) or 255 nm (AP) using a UVIKON 941 spectrophotometer. VC or AP Unidirectional mucosal to serosal fluxes (Jms) were determined during the steady state period (60-120 min).

Possible relationships between transepithelial electrical conductance ($G_t$) and VC or AP fluxes were studied by using the generate and simulate curves function of the Graphpad Prism 5 software (San Diego, CA, USA).

The flux J was calculated using: $\mathbf{J}= \frac{Qdt}{Sdt}$ (1)

where Q is the amount transported, t is time, and S is the exposed surface area (1 $cm^2$).

Cumulative permeation (Qt) was computed from:

$$\mathbf{Q_t}= Vp\left(\sum_{n=0}^{n} V - 1\right)+\mathbf{V_r C_n} \quad (2)$$

where Vp is the sampled volume, Vr the receiver compartment volume, and $C_n$ the measured concentrations [14 15].

Relationships between Gt and fluxes were analyzed using GraphPad Prism 5 (San Diego, CA, USA).

### 2.6. Tissue processing and histopathological staining procedures

Jejunum samples were collected immediately after permeability studies and fixed in 10% phosphate buffered formalin. Tissues were processed, embedded in paraffin, sectioned with a microtome, and stained with Hemalun, Phloxine, and Safran *(HPS).* Slides were examined by light microscopy, and images were recorded using Calopix software (TRIBVN SAS, France).

### 2.7. Statistics

Data are expressed as mean ± standard error (SE), with *n* representing the number of tissues from at least three rats. Statistical analysis was performed using one way ANOVA, followed by Dunnetts post hoc test (GraphPad Prism 5). $P < 0.05$ was considered statistically significant.

## 3. RESULTS

### 3.1. Effect of Physiological Media on Transepithelial Electrical Conductance and Tissue Viability

The transepithelial electrical conductance (Gt) of the jejunum was strongly influenced by the composition of the physiological media (Fig. 1A). In our experiments, Gt values ranged between 15 and 50 mS/$cm^2$. Significant differences in conductance were observed when the

mucosal and serosal compartments contained different media (Fig. 1B), except in experiments using Ringers solution (M1), PBS (M4), or DMEM (M7), where conductance remained stable.

When oral rehydration solution (M5) was added to the serosal compartment, its lower osmolarity caused a pronounced increase in conductance, from 31 to 79 $mS/cm^2$.

To better understand how medium composition affected Gt, jejunal segments were examined histologically after two hours in Ussing chambers. Clear morphological alterations were observed. As expected, pretreatment with 2% KCl resulted in complete destruction of villi while preserving the crypts [16] [17]. A similar pattern though less severe was seen with $Na^+$ free Ringer (M2) and Sorensen solution (M3), both of which induced marked villus deterioration (Fig. 2).

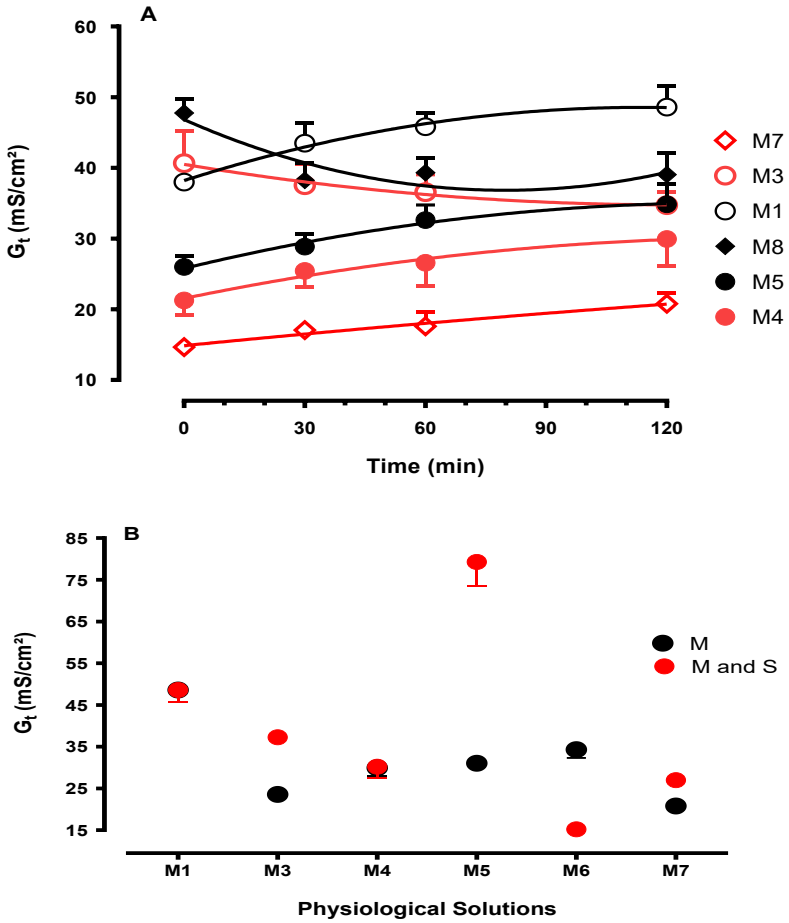


**Figure 1:** Modification of transepithelial electrical conductance $G_t$ (in $mS/cm^2$) induced by physiological media (accordingly to table 1 see A). Two conditions were illustrated in B, 1) with closed black dots the nature of the medium was changed only in mucosal (M) compartment of Ussing chamber while standard Ringer solution ~~in~~ placed in the serosal side (S), 2) with closed red dots, the same solution is placed on both sides (mucosal and serosal). The total conductance ($G_t$) of the tissue went through significant changes, except with M1 (Ringer), $M_4$ (PBS solution) and $M_7$ (Dulbecco's Modified Eagle Medium).

**Effect of different physiological media on tissue conservation**

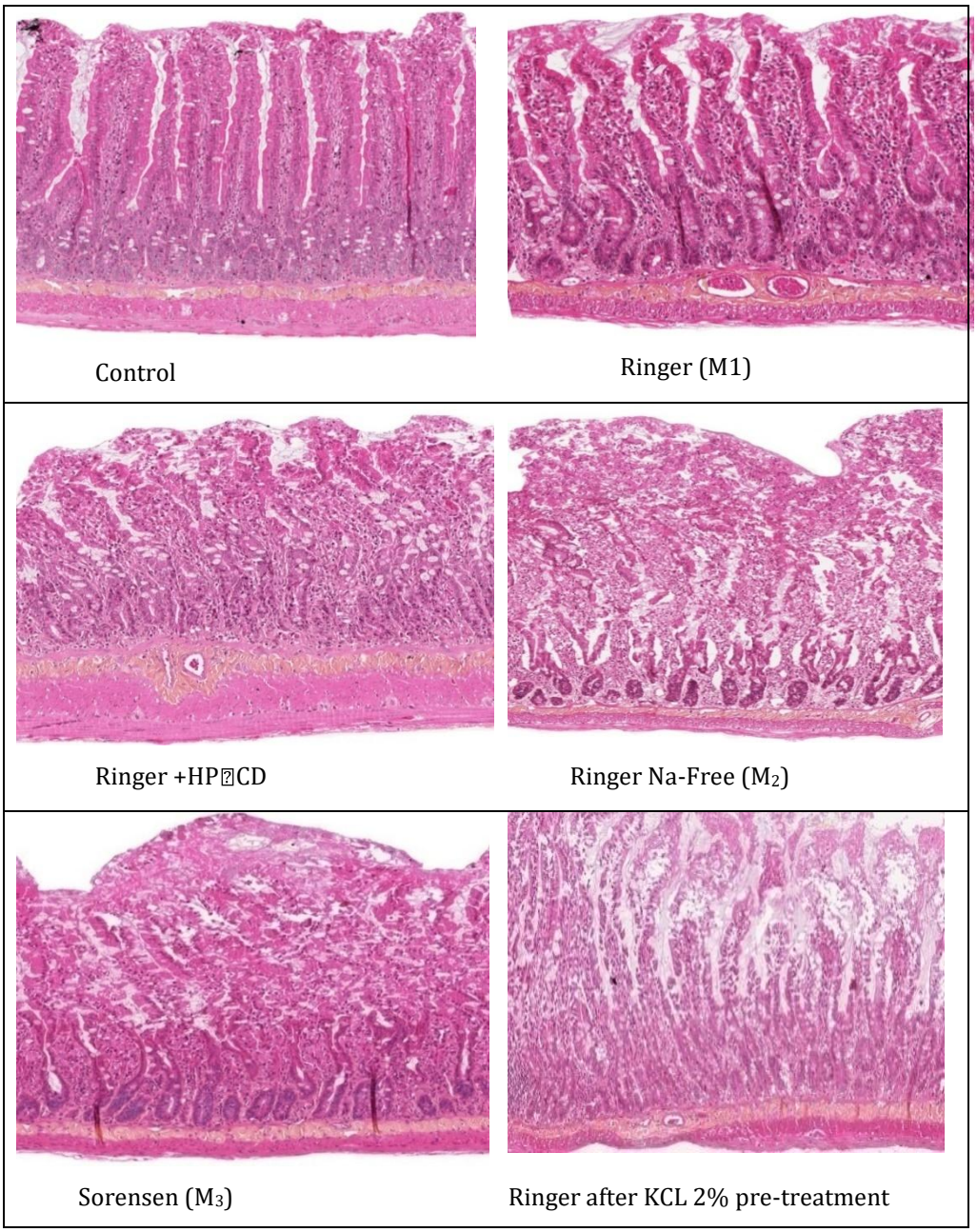


**Figure 2**: Morphology of the rat jejunum mucosa after 2 hours of incubation *in vitro* in Ussing Chambers and in presence of different physiological solutions (see table1. HPS staining, Magnification x 10. Image was treated using Calopix program (TRIBVN, SAS, France). Control microphotograph showed the whole-thickness of the jejunum mucosa without incubation in Ussing Chamber whereas $M_1$ represented the same tissue incubated with Ringer solution (positive control). Pre-treatment of tissue with KCL 2% 30 minute before utilisation induced the destruction of the villi while the crypts seemed intact. The same observation was made after 2 hours of incubation of Ringer Na-free medium ($M_2$), although these effects were reduced.

### 3.2. *In vitro* jejunal permeation of vitamin C is affected by physiological medium composition.

Unidirectional mucosal to serosal fluxes of vitamin C (VC) varied widely depending on the mucosal medium used, while Ringers solution (M1) remained constant on the serosal side. As shown in Fig. 3A, VC fluxes reached steady state after approximately 30 minutes across all media. However, the absolute flux values differed substantially by more than threefold, depending on the medium. Sorensen solution (M3) produced the highest VC transport, with Jms = 53.1 ± 8.53 $\mu g \cdot h^{-1} \cdot cm^{-2}$, compared with 31.4 ± 1.22 $\mu g \cdot h^{-1} \cdot cm^{-2}$ for control Ringer (M1). In contrast, $Na^+$ free Ringer (M2) exhibited the lowest flux, Jms = 16.0 ± 1.53 $\mu g \cdot h^{-1} \cdot cm^{-2}$ (Fig. 3B).

A strong linear correlation ($r^2$ = 0.98) was observed between transepithelial conductance and VC fluxes (Fig. 3C), indicating that increased ionic permeability is associated with enhanced vitamin C transport.

**Table 2: Conductance variation**

| Modification of physiological medium physiological medium in mucosal side | | | |
|---|---|---|---|
| **Mucosal side** | **Serosal side** | **$G_t$ (mS/cm²)** | **P value** |
| $M_1$ | $M_1$ | 48.60 ± 7.70 (n=7) | Control |
| $M_3$ | $M_1$ | 34.72 ± 5.80 (n=9) | p <0.01 |
| $M_3$+HPCD | $M_1$ | 23.58 ± 3.33 (n=7) | P < 0.01 |
| $M_5$+HPCD | $M_1$ | 31.03 ± 4.60 (n=9) | P <0.01 |
| $M_6$+HPCD | $M_1$ | 34.31 ± 6.00 (n=9) | P < 0.01 |
| $M_7$ | $M_1$ | 20.80 ± 4.60 (n=8) | P < 0.01 |
| $M_7$ + HPCD | $M_1$ | 44.67 ± 4.80 (n=8) | P >0.05 |
| Conservation of the same medium on both sides | | | |
| Mucosal side | Serosal side | $G_t$ (mS/cm²) | P value |
| $M_5$ | $M_5$ | 79.29 ± 18.07 (n=10) | P<0.01 |
| $M_5$+HPCD | $M_5$ | 80.45 ± 13.18 (n=8) | P<0.01 |
| $M_7$ | $M_7$ | 26.98 ± 3.60 (n=9) | P<0.01 |
| $M_4$+HPCD | $M_4$ | 29.95 ± 10.72 (n=8) | P<0.01 |
| $M_3$+HPCD | $M_3$ | 37.25 ± 4.70 (n=7) | p>0.05 |
| $M_8$+Glu | $M_8$+Glu | 39.05 ± 8.04 (n=7) | p>0.05 |
| $M_6$ | $M_6$ | 34.86 ± 7.53 (n=7) | p>0.05 |
| $M_6$+HPCD | $M_6$ | 15.20 ± 4.89 (n=8) | P<0.01 |

One way analysis of ANOVA followed by Dunnett Multiple Comparison test (GraphPad PrismVers 5.

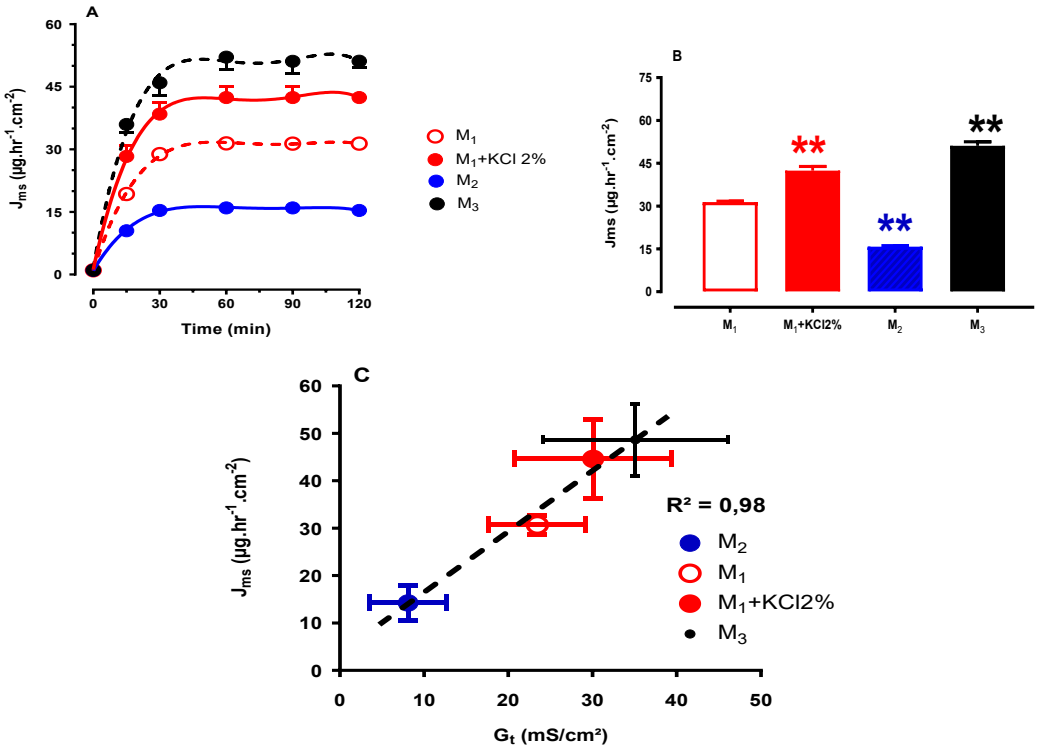


**Figure 3**: Time course fluxes of vitamin C (VC) in rat jejunum *in vitro* (fig.3A) and the fluxes of Vit C after 2 hours with a different media (fig.3B). Pre-treatment of jejunum by adding KCL 2% in on the mucosal side of the loops during 30 minutes before using was represented by $M_1$+KCL 2% whereas $M_1$ is Ringer solution, $M_2$ Ringer with sodium free, and $M_3$ Sorensen medium. **p<0.01 (ANOVA followed by Dunnett t-test). N = 8-9 tissues of 4 rats. $J_{ms}$ indicates unidirectional fluxes from mucosal (luminal) to serosal (blood) side. Fig 3C, Relationship between fluxes at equilibrium (in µg.hr$^{-1}$.cm$^{-2}$) and Transepithelial electrical Conductance $G_t$ (in mS/cm²). The data were obtained from the means of at least n = 16 tissues from 7 rats. In fig 5A, closed black dots indicate the values obtained with jejunum after 30 minutes of pre-treatment with KCL 2% and closed red dots Ringer solution with Na-free, Blue closed dots with Sorensen medium whereas open black dots with Ringer solution (or control medium). The relationship between $G_t$ and unidirectional mucosal to serosal fluxes (Jms) were analyzed by linear regression. $r^2$ was 0.98. The conductance (an index of ionic permeability) increased due to an increase of vitamin C fluxes.

### 3.3. *In vitro* jejunal permeation of acetaminophen is affected by physiological medium composition.

As with vitamin C, acetaminophen (AP) fluxes stabilized after approximately 30 minutes (Fig. 4A). AP transport was significantly lower when Sorensen solution (M3) was used, yielding Jms = 12.63 ± 1.99 $\mu g \cdot h^{-1} \cdot cm^{-2}$, compared with 17.86 ± 1.39 $\mu g \cdot h^{-1} \cdot cm^{-2}$ in Ringer's solution (M1) (Fig. 4B).

Despite only two experimental datasets being available, no clear linear relationship was found between Gt and AP fluxes (Fig. 4C). Even when conductance values were similar (~30 $mS/cm^2$), AP fluxes differed markedly between M1 and M3. This suggests that AP transport may be regulated by factors other than passive ionic permeability, such as interactions with efflux transporters.

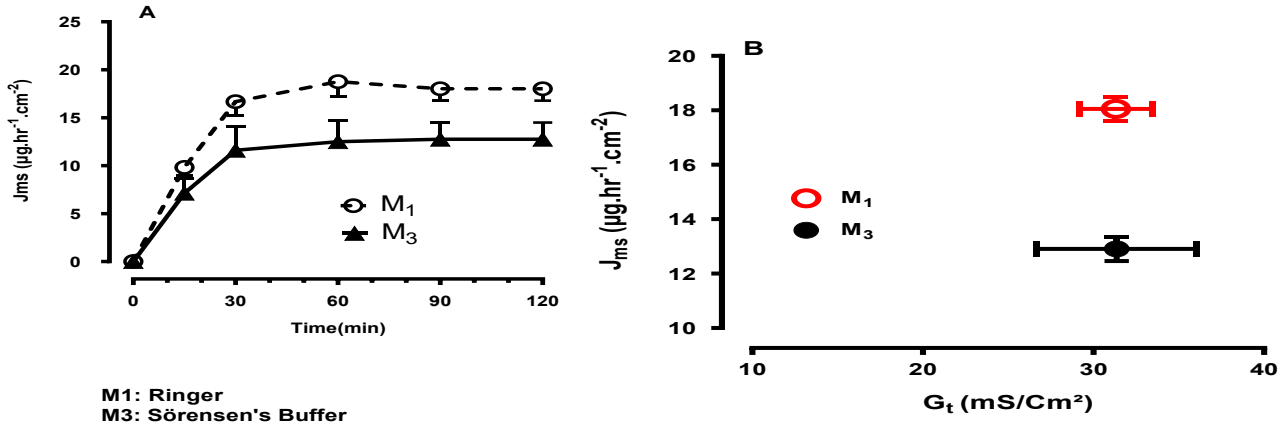


**Figure 4**: Time course fluxes of acetaminophen (AP) across rat jejunum *in vitro* (fig.4A) and fluxes of AP after 2 hours with different mediums (fig.4B). $M_2$ is Ringer solution whereas $M_3$ is Sorensen solution. *p<0.05 (ANOVA and T-test). n= 12 tissues of 4 rats. $J_{ms}$ indicates unidirectional fluxes from mucosal (luminal) to serosal (blood) side. In fig 4C, open black dots indicate the values obtained with the Ringer solution ($M_1$) and green closed dots with the Sorensen ($M_3$) medium. With transepithelial electrical, Conductance was closed ~~to~~ at 30 $mS/cm^2$, the unidirectional fluxes ($J_{ms}$) of AP was different in $M_1$ and $M_3$.

### 3.4. Tissue Functional Viability: Effects of Medium Composition on Responses to Pharmacological Stimuli

The functional viability of jejunal tissue was assessed through its electrical responses to glucose (30 mM) and carbacholine ($10^{-4}$ M). Representative short circuit current (Isc) recordings are shown in Fig. 5.

- In $Na^+$ free Ringer (M2), no response to either glucose or carbacholine was detected, indicating severe impairment of sodium dependent transport mechanisms.
- In Sorensen solution (M3), both responses were present but substantially reduced relative to Ringer (M1).
- In Ringer (M1), tissues displayed the expected glucose stimulated increase in Isc and a robust carbacholine induced chloride secretion.

Overall, these findings demonstrate that the ionic composition of the medium profoundly influences both epithelial integrity and functional activity, affecting nutrient absorption and secretory capacity.

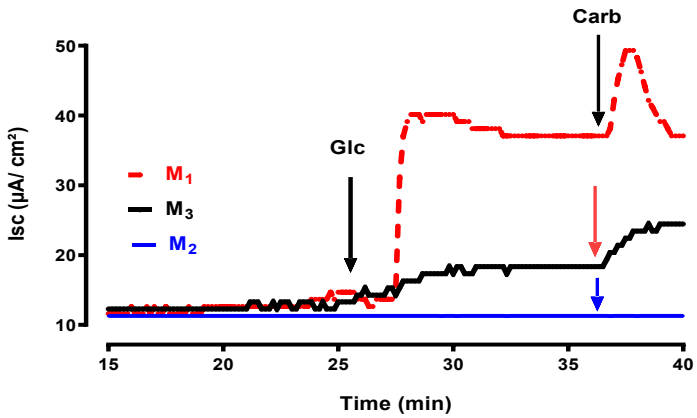


**Figure 5:** Typical recording of the short-circuit current intensity showing the impact of physiological medium on glucose (Glc 30 mM) absorption and chloride secretion induced by carbacholine ($10^{-4}$M). Ringer solution ($M_1$), $Na^+$-free-Ringer ($M_2$), Sorensen medium ($M_3$).

## 4. DISCUSSION

*In vitro* studies of intestinal absorption of drugs are very useful during preclinical development. Experimental models such as Ussing chambers attempt to reproduce what happens in vivo, but they clearly cannot replace *in vivo* studies in whole animals, where many additional known or unknown parameters are involved. The aim of the present work was to investigate the **impact** of the composition of physiological media used during Ussing chamber experiments.

The present study confirmed that the integrity of the proximal jejunum, when incubated in Ussing chambers, begins to decline after two hours and **exhibits** morphological deterioration of the epithelial villi, even though the crypt structures remain intact [18] [19]. Furthermore, it showed that some commonly used media for *in vitro* studies can affect the functional viability of the tissue and the experimentally measured drug fluxes through the intestinal mucosa. Therefore, if any changes in the composition of physiological media are planned, these must be taken into account before performing *in vitro* drug permeability studies.

It is generally accepted that the incubation of tissue in Ussing chambers cannot exceed four hours due to progressive tissue deterioration. The present study confirmed the observations reported by Inagaki Tachibana et al. (Inagaki Tachibana et al., 2008), with the important difference that this deterioration also depends on the composition of the physiological medium used. In this study, tissue deterioration was more pronounced with Na free Ringer (M2) and Sorensen buffer (M3), whereas Ringer's solution (M1) caused less damage. In addition, the study showed that morphological modifications of the tissue were associated with changes in transmural electrical conductance (Gt), an index of passive permeability to ions and small electrolytes, mainly NaCl.

This study highlighted the crucial role of physiological medium composition on: (a) the integrity of the tissue during incubation in Ussing chambers, and (b) transmural electrical conductance (Gt), i.e., passive and electrogenic ion transport processes, with possible implications for the transport of drugs, nutrients, and xenobiotics coupled to $Na^+$, such as glucose, vitamin C (VC), and short amino acids. We found that modifying the physiological media during Ussing chamber studies altered the unidirectional fluxes of vitamin C and acetaminophen (AP).

After oral administration, VC is absorbed from the intestinal lumen and released into the bloodstream. In the gastrointestinal tract, the ionized form of VC, ascorbate (ASC), and its oxidized counterpart, dehydroascorbic acid (DHA), are absorbed through different transporters, with higher affinity for ASC than for DHA, following a Michaelis–Menten rate, where saturation reflects increases in substrate concentration [20] [21]. Because they are ionized at physiological pH, ASC and DHA do not easily cross biological membranes and primarily depend on transporters located in the cell membrane [22] [23]. However, both molecules can cross passively to a small extent. It has also been suggested that during intestinal uptake and renal reabsorption, ASC exits epithelial cells from the basolateral side down its concentration gradient by passive diffusion [24].

Our study showed that when sodium was removed from the medium (M2), unidirectional vitamin C fluxes decreased by half but were not entirely suppressed, confirming both the sodium dependent uptake of VC and its passive transport component. In addition, when transmural electrical conductance (Gt) increased with Sorensen medium (M3), the amount of vitamin C crossing the intestinal epithelium also increased, as expected from the strong linear correlation ($r^2$ = 0.98) between fluxes and Gt. Two specific transporters, SVCT1 and SVCT2, previously described by Tsukaguchi et al., enable active transport of ASC against a concentration gradient, allowing intracellular accumulation reaching concentrations more than 50 fold higher than extracellular levels [25] [26]. Sodium dependency of this transport has a stoichiometry of two $Na^+$ ions for one ASC anion [27], demonstrating a secondary active transport mechanism maintained by sodium/potassium ATPase.

We also evaluated the passage of acetaminophen through rat jejunum incubated in Ussing chambers with Ringer's solution or Sorensen buffer. In this case, we observed that with the same transmural conductance (Gt), unidirectional AP fluxes differed, suggesting little or no involvement of passive transport processes. Novak et al. [9] demonstrated that AP inhibits Pgp activity and increases intestinal absorption of digoxin, a prototypical substrate. In our study, AP intestinal bioavailability decreased with Sorensen buffer compared with Ringer's solution. This reduction in AP flux may be attributed to changes in intestinal morphology or efflux transporter activity. Schafer et al. [28] reported that AP modified apical cell surface structures, reducing the number of microvilli and altering membrane properties through different mechanisms. They showed that this reduced permeability to small molecules and increased MDR1 efflux activity. We also observed that Sorensen buffer alters intestinal villi when incubated in Ussing chambers, suggesting that both AP and M3 effects may reduce AP bioavailability through mucosal alterations and increased efflux transporter activity.

Modification of the composition of physiological media used in Ussing chamber experiments may therefore strongly influence tissue responses to effectors involved in electrogenic transport processes or affect functional tissue viability. A key example is shown in Figure 6. Suppression of sodium in Ringers solution completely eliminated tissue responses to glucose stimulation and carbacholine, while reducing sodium availability decreased tissue responses.

In conclusion, this study confirms that the proximal jejunum incubated in Ussing chambers shows progressive morphological deterioration, mainly in the villi of epithelial cells, even though the crypt structure remains intact. The study also shows that some commonly used media can affect tissue functional

viability and drug permeability across the intestinal mucosa, suggesting that the composition of the physiological medium must be carefully considered in *in vitro* intestinal bioavailability studies.

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## REFERENCES

1. Pélissier MA, Vasquez N, Balamurugan R, et al. Metronidazole effects on microbiota and mucus layer thickness in the rat gut. FEMS Microbiol Ecol. 2010;73(3):601-610. https://doi.org/10.1111/j.1574-6941.2010.00916.x PMid:20579100

2. Pappenheimer JR. Role of Pre-epithelial ''Unstirred'' Layers in Absorption of Nutrients from the Human Jejunum. The Journal of membrane biology. 2001;179:185-204. https://doi.org/10.1007/s002320010047 PMid:11246419

3. Fairstein M, Swissa R, Dahan A. Regional-Dependent Intestinal Permeability and BCS Classification: Elucidation of pH-Related Complexity in Rats Using Pseudoephedrine. AAPS J. 2013;15(2):589-597. https://doi.org/10.1208/s12248-013-9462-x PMid:23440549 PMCid:PMC3675759

4. Dossou-Yovo F, Mamadou G, Soudy ID, et al. Metronidazole or Cotrimoxazole therapy is associated with a decrease in intestinal bioavailability of common antiretroviral drugs. PLoS One. 2014;9(2):e89943. https://doi.org/10.1371/journal.pone.0089943 PMid:24587140 PMCid:PMC3935968

5. Swaan PW, Marks GJ, Ryan FM, Smith PL. Determination of transport rates for arginine and acetaminophen in rabbit intestinal tissues in vitro. Pharm Res. 1994;11(2):283-287. https://doi.org/10.1023/A:1018967727156 PMid:8165189

6. Ungell AL, Nylander S, Bergstrand S, Sjöberg Å, Lennernäs H. Membrane Transport of Drugs in Different Regions of the Intestinal Tract of the Rat. Journal of Pharmaceutical Sciences. 1998;87(3):360-366. https://doi.org/10.1021/js970218s PMid:9523990

7. Bürzle M, Suzuki Y, Ackermann D, et al. The sodium-dependent ascorbic acid transporter family SLC23. Mol Aspects Med. 2013;34(2-3):436-454. https://doi.org/10.1016/j.mam.2012.12.002 PMid:23506882

8. Neirinckx E, Vervaet C, Michiels J, et al. Feasibility of the Ussing chamber technique for the determination of in vitro jejunal permeability of passively absorbed compounds in different animal species. J Vet Pharmacol Ther. 2011;34(3):290-297. https://doi.org/10.1111/j.1365-2885.2010.01218.x PMid:21492193

9. Novak A, Carpini GD, Ruiz ML, et al. Acetaminophen inhibits intestinal p-glycoprotein transport activity. J Pharm Sci. 2013;102(10):3830-3837. https://doi.org/10.1002/jps.23673 PMid:23897240

10. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes Text with EEA Relevance. Vol 276. 2010. Accessed March 20, 2026. http://data.europa.eu/eli/dir/2010/63/oj

11. Mamadou G, Charrueau C, Dairou J, Limas Nzouzi N, Eto B, Ponchel G. Increased intestinal permeation and modulation of presystemic metabolism of resveratrol formulated into self-emulsifying drug delivery systems. International Journal of Pharmaceutics. 2017;521(1-2):150-155. https://doi.org/10.1016/j.ijpharm.2017.02.036 PMid:28216465

12. Ayed FB, Mamadou G, Mrabti HN, Limas-Nzouzi N, Eto B, Saguem S. Studies on intestinal passage of flumequine and oxytetracycline-loaded MIL-100 (Fe) in the presence of divalent ions. Trop J Pharm Res. 2018;17(7):1295. https://doi.org/10.4314/tjpr.v17i7.10

13. Myers RN, Brown CE, Deaver JM. In vivo effect of potassium on small bowel. Ann Surg. 1967;166(4):693-703. D https://doi.org/10.1097/00000658-196710000-00016 PMid:6061547 PMCid:PMC1477435

14. Brodin B, Steffansen B, Nielsen C. Passive diffusion of drug substances: The concepts of flux and permeability. In: 2010:135-151.

15. Mbah CJ, Nnadi CO. Transdermal Delivery of Gabapentin: Effect of Cosolvent and Microemulsion on Permeation through the Rat Skin. Pharmacology & Pharmacy. 2014;5(5):471-478. https://doi.org/10.4236/pp.2014.55057

16. Clarke LL. A guide to Ussing chamber studies of mouse intestine. Am J Physiol Gastrointest Liver Physiol. 2009;296(6):G1151-1166. https://doi.org/10.1152/ajpgi.90649.2008 PMid:19342508 PMCid:PMC2697950

17. Caraballo JC, Yshii C, Butti ML, et al. Hypoxia increases transepithelial electrical conductance and reduces occludin at the plasma membrane in alveolar epithelial cells via PKC-ζ and PP2A pathway. Am J Physiol Lung Cell Mol Physiol. 2011;300(4):L569-578. https://doi.org/10.1152/ajplung.00109.2010 PMid:21257729 PMCid:PMC3075095

18. Inagaki E, Natori Y, Ohgishi Y, Hayashi H, Suzuki Y. Segmental difference of mucosal damage along the length of a mouse small intestine in an Ussing chamber. J Nutr Sci Vitaminol (Tokyo). 2005;51(6):406-412. https://doi.org/10.3177/jnsv.51.406 PMid:16521699

19. Inagaki-Tachibana E, Natori Y, Hayashi H, Suzuki Y. In vitro diffusion barriers of the mouse jejunum in Ussing chambers. J Nutr Sci Vitaminol (Tokyo). 2008;54(1):30-38. https://doi.org/10.3177/jnsv.54.30 PMid:18388405

20. Malo C, Wilson JX. Glucose Modulates Vitamin C Transport in Adult Human Small Intestinal Brush Border Membrane Vesicles. The Journal of Nutrition. 2000;130(1):63-69. https://doi.org/10.1093/jn/130.1.63 PMid:10613768

21. Lindblad M, Tveden-Nyborg P, Lykkesfeldt J. Regulation of Vitamin C Homeostasis during Deficiency. Nutrients. 2013;5(8):2860-2879. https://doi.org/10.3390/nu5082860 PMid:23892714 PMCid:PMC3775232

22. Rose RC. Solubility properties of reduced and oxidized ascorbate as determinants of membrane permeation. Biochim Biophys Acta. 1987;924(1):254-256. https://doi.org/10.1016/0304-4165(87)90094-8

23. Wilson JX, Dixon SJ. High-affinity sodium-dependent uptake of ascorbic acid by rat osteoblasts. J Membr Biol. 1989;111(1):83-91. https://doi.org/10.1007/BF01869211 PMid:2810353

24. Goldenberg H, Schweinzer E. Transport of vitamin C in animal and human cells. J Bioenerg Biomembr. 1994;26(4):359-367. https://doi.org/10.1007/BF00762776 PMid:7844110

25. Welch RW, Bergsten P, Butler JD, Levine M. Ascorbic acid accumulation and transport in human fibroblasts. Biochem J. 1993;294 ( Pt 2)(Pt 2):505-510.

https://doi.org/10.1042/bj2940505 PMid:8373364 PMCid:PMC1134483

26. Tsukaguchi H, Tokui T, Mackenzie B, et al. A family of mammalian Na+-dependent L-ascorbic acid transporters. Nature. 1999;399(6731):70-75. https://doi.org/10.1038/19986 PMid:10331392

27. Maffia M, Ahearn GA, Vilella S, Zonno V, Storelli C. Ascorbic acid transport by intestinal brush-border membrane vesicles of the teleost Anguilla anguilla. Am J Physiol. 1993;264(6 Pt 2):R1248-1253. https://doi.org/10.1152/ajpregu.1993.264.6.R1248 PMid:8322981

28. Schäfer C, Schröder KR, Höglinger O, Tollabimazraehno S, Lornejad-Schäfer MR. Acetaminophen changes intestinal epithelial cell membrane properties, subsequently affecting absorption processes. Cell Physiol Biochem. 2013;32(2):431-447. https://doi.org/10.1159/000354449 PMid:23988609