# Hot topic E: Permeability: From in vitro best practices to in vivo relevance

**“Measurement of the membrane permeability to drug candidates has helped reduce the pharmacokinetically related drug attrition rates from 40% to 10%”**


**1**.Chipot C in: Predictions from First-Principles of Membrane Permeability to Small Molecules: How Useful Are They in Practice? J Chem Inf Model. 2023 Aug 14;63(15):4533-4544.
**2**.KolaI,LandisJ.Canthepharmaceuticalindustryreduceattritionrates?NatRevDrugDiscov.2004;3:711–716.
**3**.LipinskiCA,LombardoF,DominyBW,FeeneyPJ.ExperimentalandcomputationalapproachestoestimatesolubilityandpermeabilityindrugdiscoveryanddevelopmentsettingsAdvDrugDelivRev.2001;46(1-3):3–26.
**4**.VolpeDA.Applicationofmethodsuitabilityfordrugpermeabilityclassification.AAPSJ.2010;12(4):670–678.



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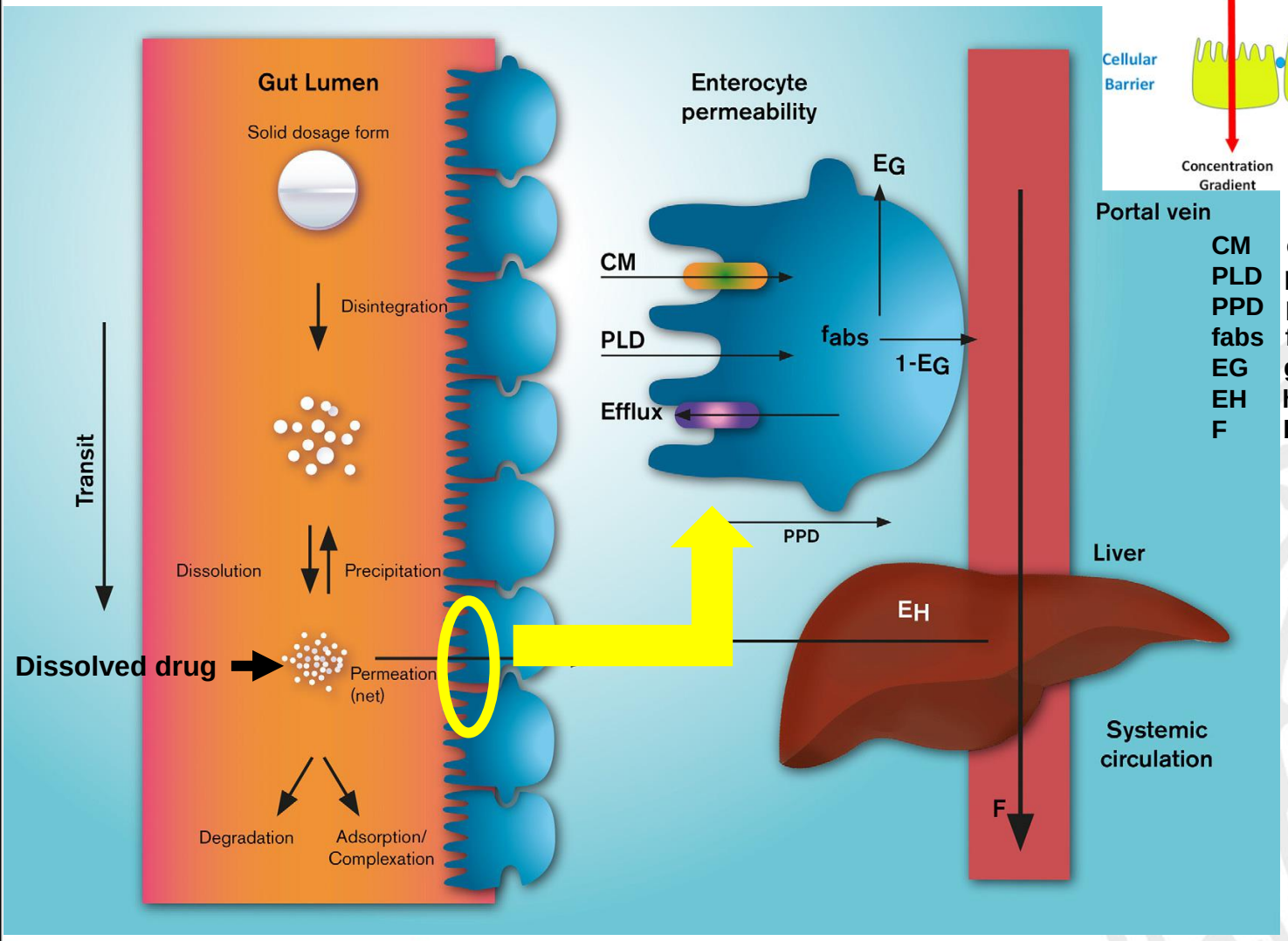
**Sweden**

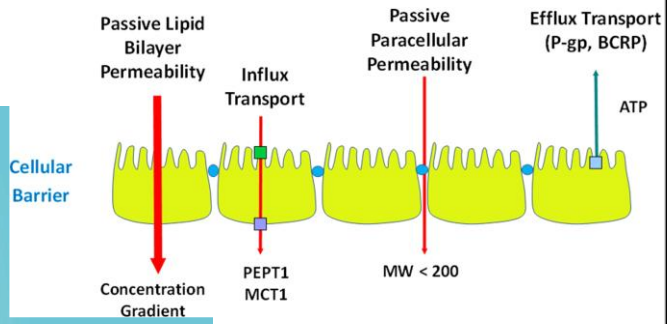

# Outline of Today's lecture

- Human small and large intestinal permeability is the starting point –provides *in vivo* relevance for excisting non-clinical and *in vitro* models as well as future more intestinal *in vitro* models.
- Experimental *in vitro* and non-clinical methods and comparisons for regional permeability
- Role of intestinal efflux *in vivo* and model predictions
- Future *In vitro* models with improved accuracy and precision?


1. Doluisio JT, Billups NF, Dittert LW, Sugita ET, Swintosky JV. Drug absorption. I. An in situ rat gut technique yielding realistic absorption rates. J Pharm Sci. 1969;58(10):1196–200.
2. Doluisio JT, Tan GH, Billups NF, Diamond L. Drug absorption. II. Effect of fasting on intestinal drug absorption. J Pharm Sci. 1969;58(10):1200–2.
3. Ochsenfahrt H, Winne D. Der Einfluss des Wassernettofluxes auf die Resorption von Arzneimitteln [Influence of net flux of water on that absorption of drugs]. Naunyn Schmiedebergs Arch Pharmakol. 1970;266(4):414-5. German. PMID: 4253840.
4. Fogh, J., J.M. Fogh, and T.J.J.o.t.N.C.I. Orfeo, One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. Journal of the National Cancer Institute. 1977.
5. Hidalgo, I., T. Raub, and R. Borchardt, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology. 1989. 96(2): p. 736-749
6. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995 Mar;12(3):413-20. doi: 10.1023/a:1016212804288. PMID: 7617530.

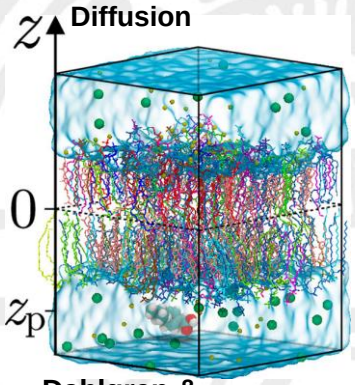
# *In vivo* relevance of *in vitro* permeability models – site of measurements




| | |
|---|---|
| CM | carrier mediated |
| PLD | passive lipoidal diffusion |
| PPD | passive paracellular diffusion |
| fabs | fraction absorbed |
| EG | gut-wall extraction |
| EH | hepatic extraction |
| F | bioavailability |

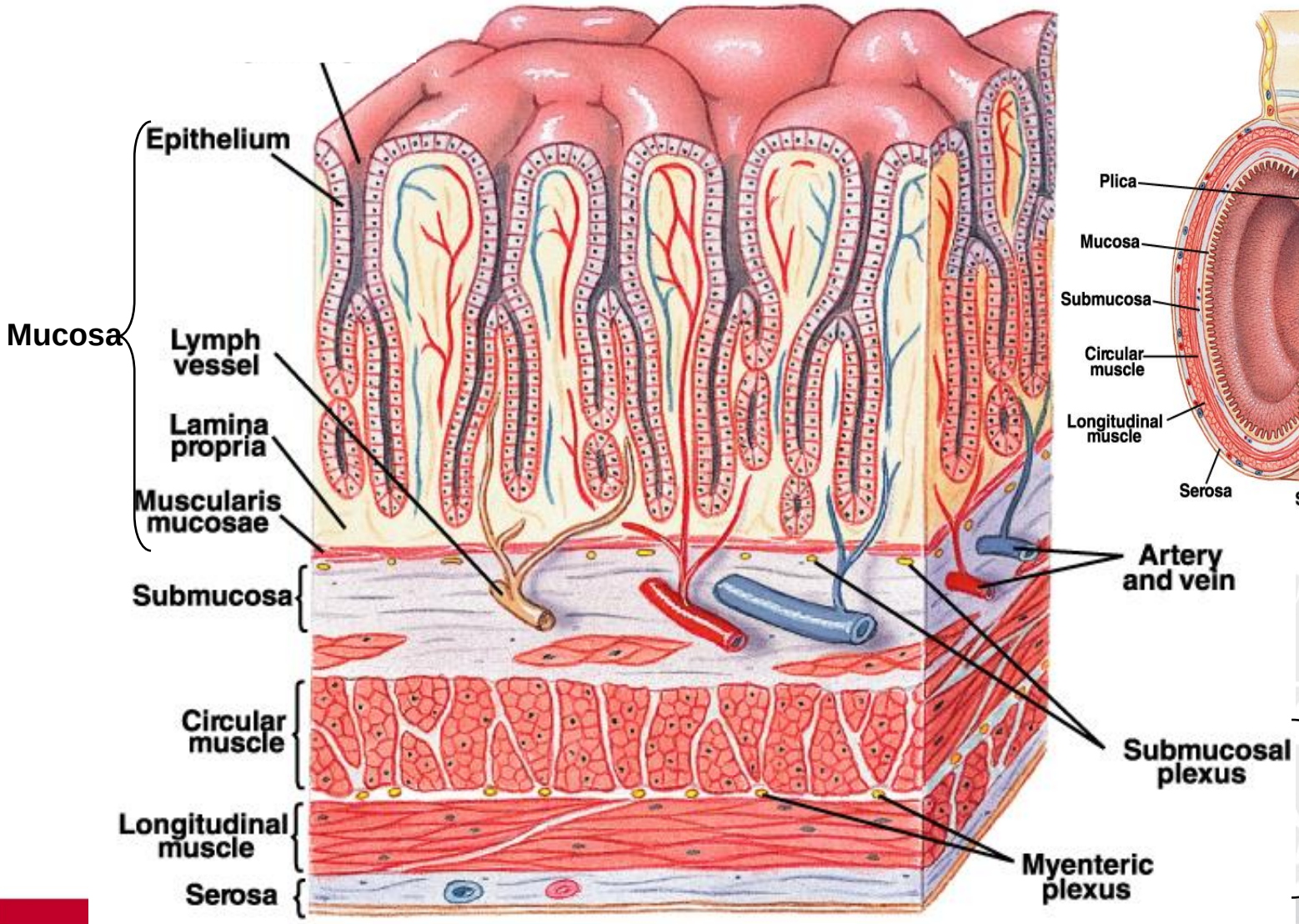
Lipidiol Diffusion = Transcellular Membrane Diffusion

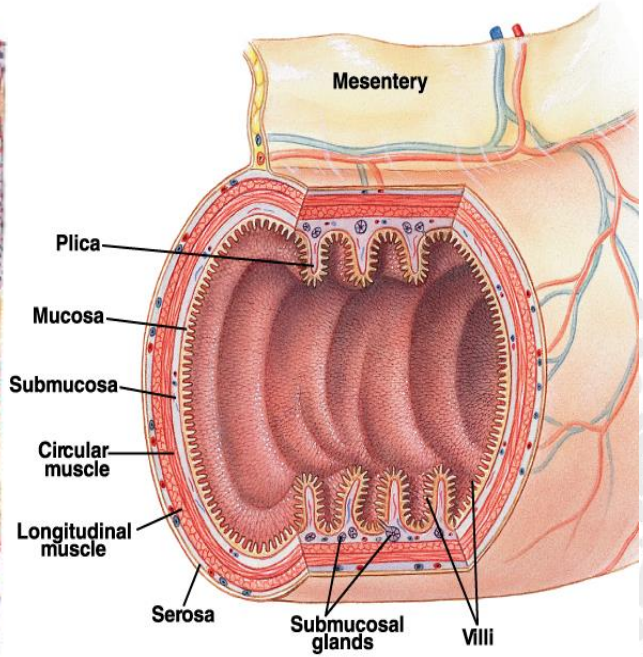



Dahlgren & Lennernäs Book Chapter 2021

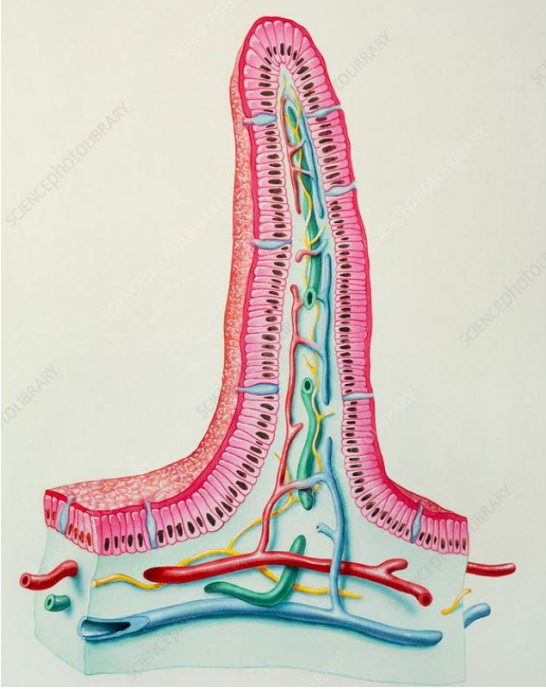
# Small intestinal barrier

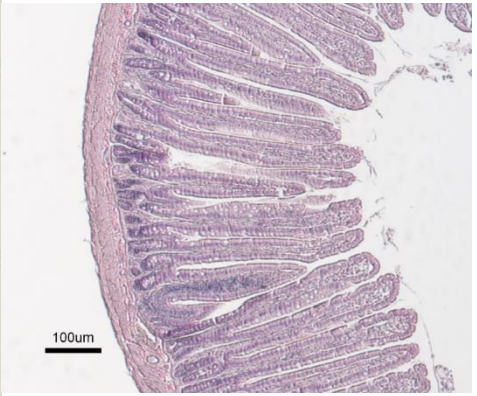
- Is organized in finger-like protrusions called villi and invaginations called crypts.
- Is a dynamic physiological barrier that is sensing and receiving feedback neuroendocrine signals that maintains the delicate balance between permeability and protective barrier functions



# Small and Large Intestinal Mucosa and site of measurements

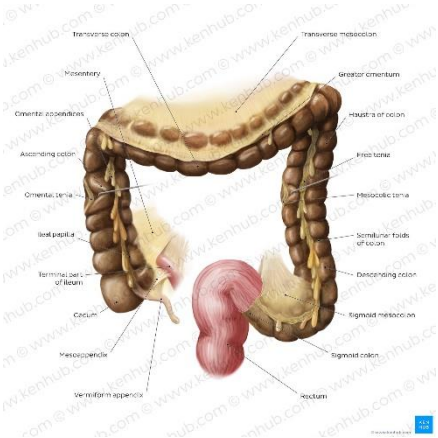





- Enterocytes
- Goblet cells
- Enteroendocrine cells
- Paneth cells

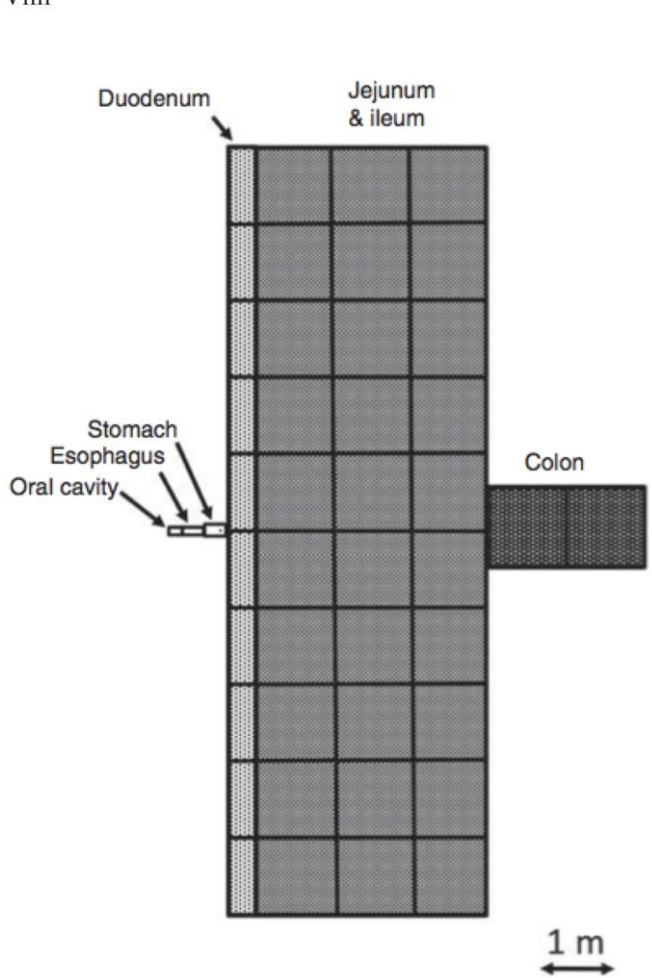

**Colon mucosa is lined by simple columnar epithelium (lamina epithelialis) with long microvilli.**

***https://www.sciencephoto.com/media/309980/view/artwork-of-a-section-through-an-intestinal-villus***

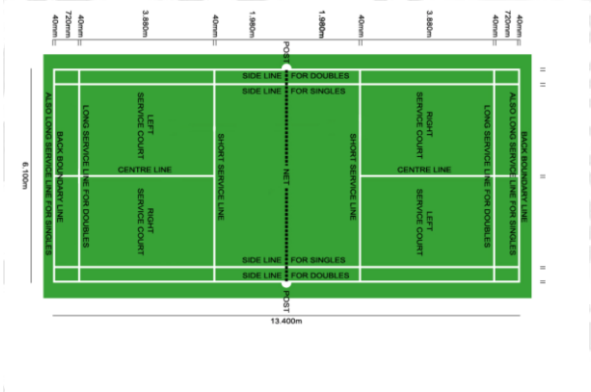



**The colon makes up the longest part of the large intestine. It begins from the caecum at the ileocecal valve and ends in the rectum.**

# Approximate anatomical distribution of the mucosal luminal surfaces along the digestive tract.



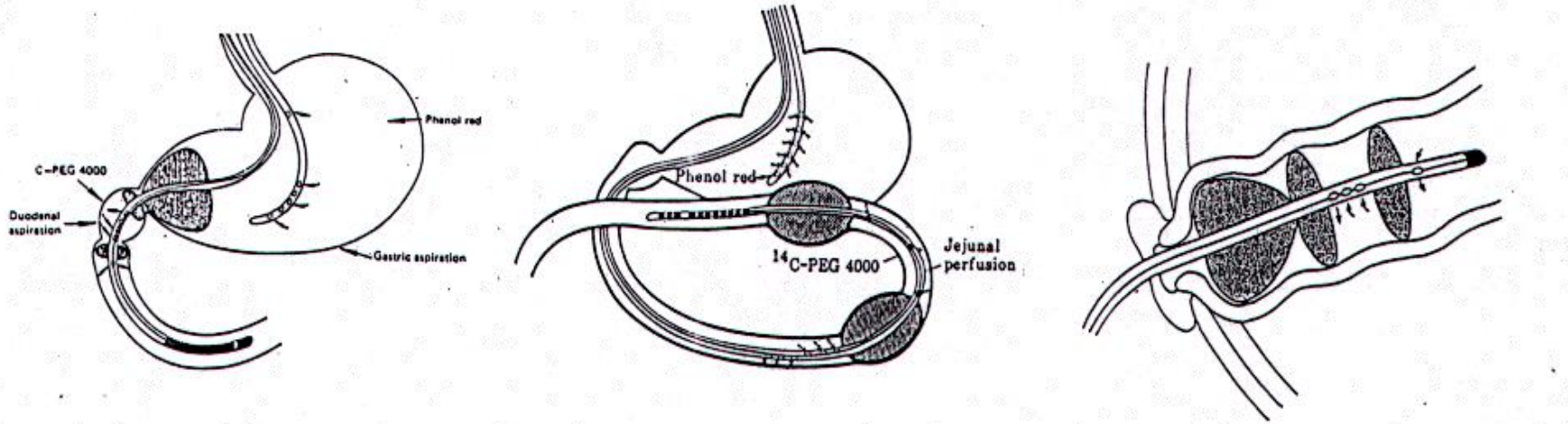

- Approximately the area (A) used to calculate Peff is 0.33 $m^2$ in the perfused jejunal segment (LOC-I-GUT)
- According to novel data the total A of the GI mucosa in adult humans is calculated to be about 30-40 $m^2$.
- "The total area of the human adult gut mucosa is not in the order of tennis lawn (300-400 $m^2$), rather is that of half a badminton court (30-40 $m^2$)".




**Helander HF & Fändriks L. Scand. J Gastroenterology. 2014; 49: 681–689**
**Surface area of the digestive tract – revisited**

# *In vivo* single-pass perfusion of jejunum and rectum in humans

## The Loc-I-GUT Concept



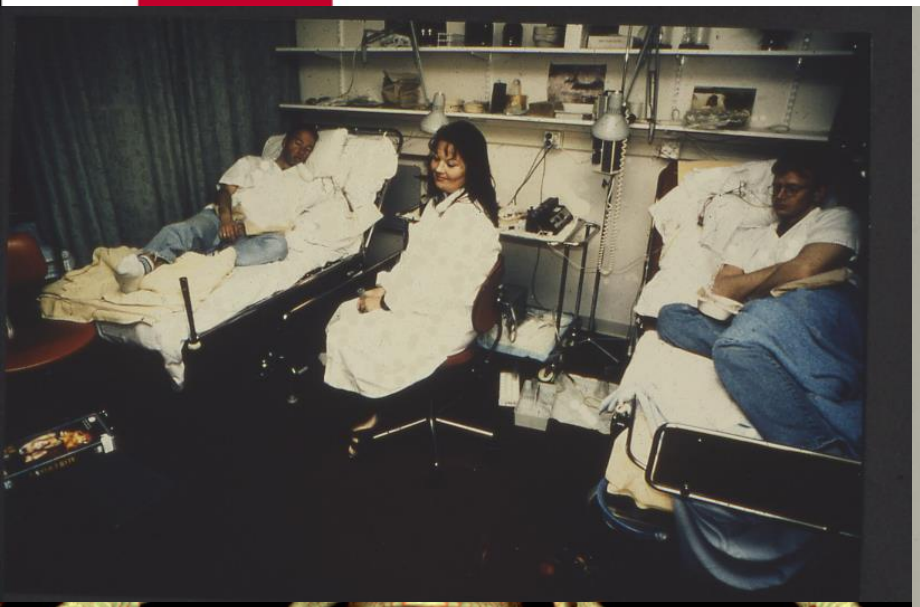


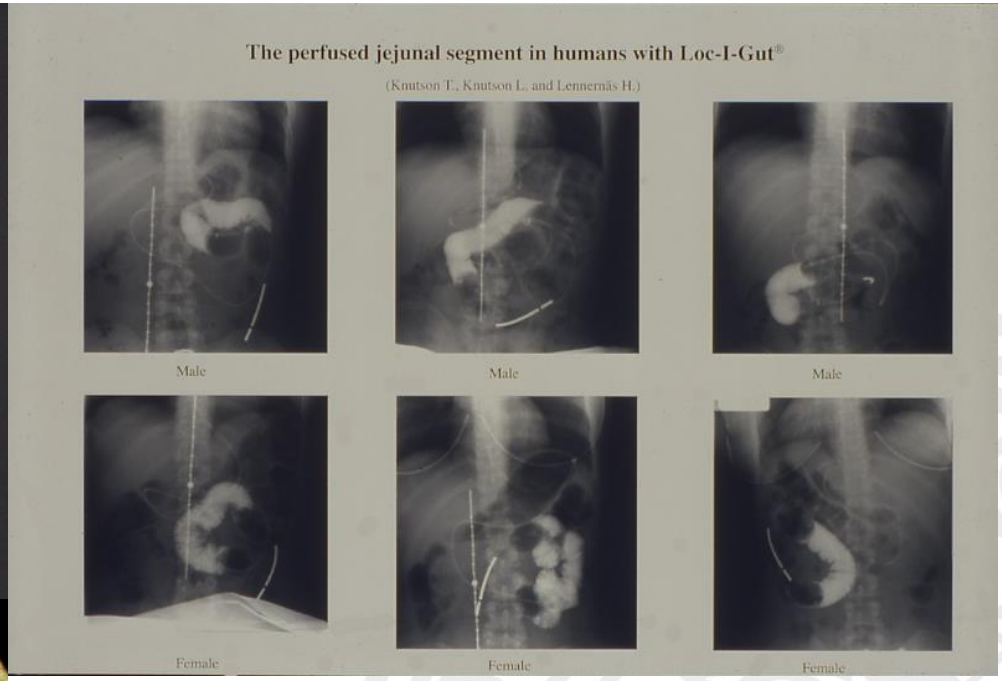
Knutson L., Odlind B. and Hällgren R. A new technique for segmental jejunal perfusion in man. Am. J. Gastroenterol. 84: 1278-1284, 1989.

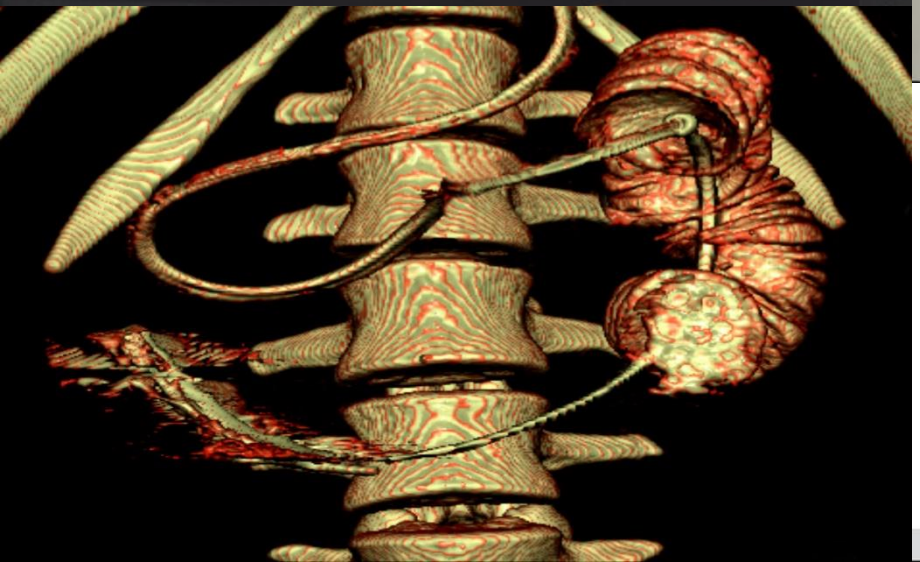
Lennernäs H., Ahrenstedt Ö., Hällgren R., Knutson L., Ryde M. and Paalzow L.K. Regional jejunal perfusion, a new *in vivo* approach to study oral drug absorption in man. Pharm. Res.9, 1243-1251, 1992.

# Clinical experiments performed *in vivo* in humans: Loc-I-Gut

Knutson T, Fridblom P, Ahlström H, Magnusson A, Tannergren C, Lennernäs H. Mol Pharm. 2009;6(1):2-10





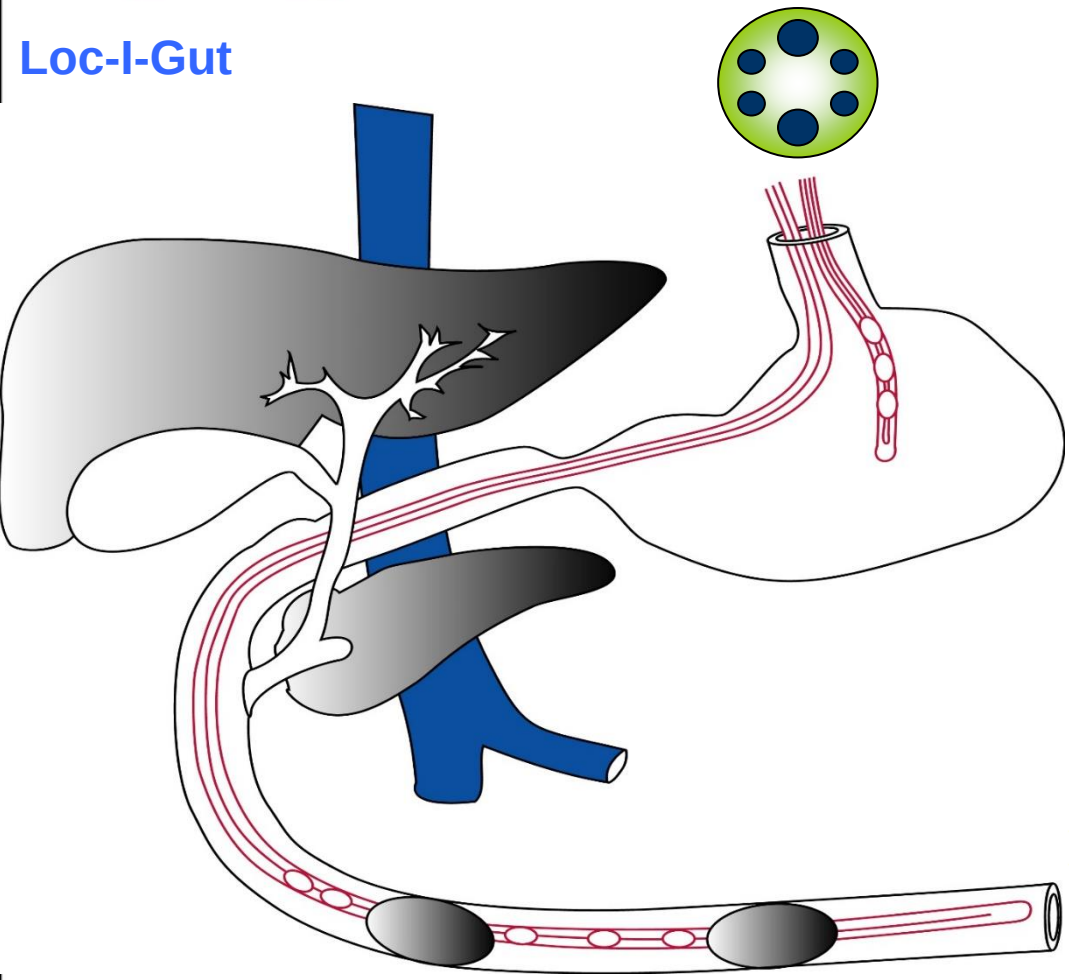



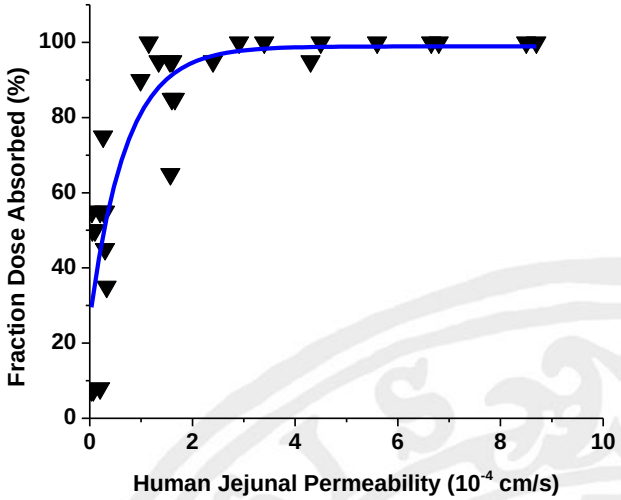
**With Multi-slice Computed Spiral Tomography**
**and air as a contrast medium a three dimensional**
**view of the perfused segment is obtained.**

# Intestinal permeability and plasma pharmacokinetic assessment simultaneously in humans (in vivo)

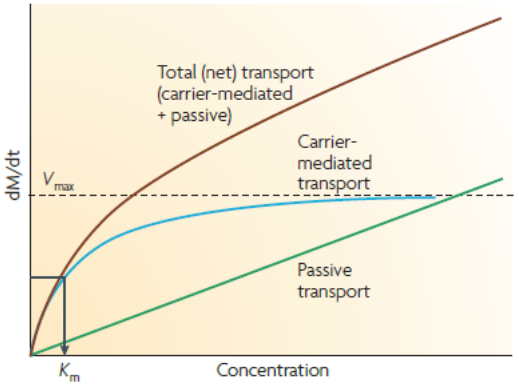
**Lennernäs H. et al, Pharm Res, 1992, 9(10), 1243-1251**

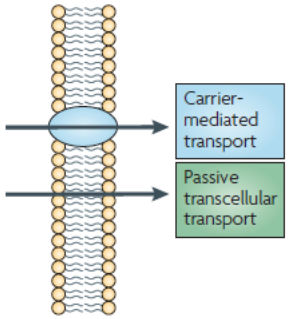
## Loc-I-Gut



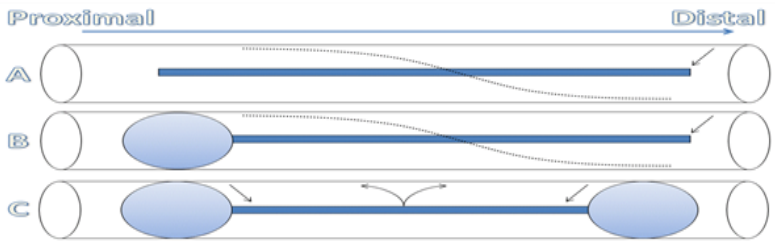


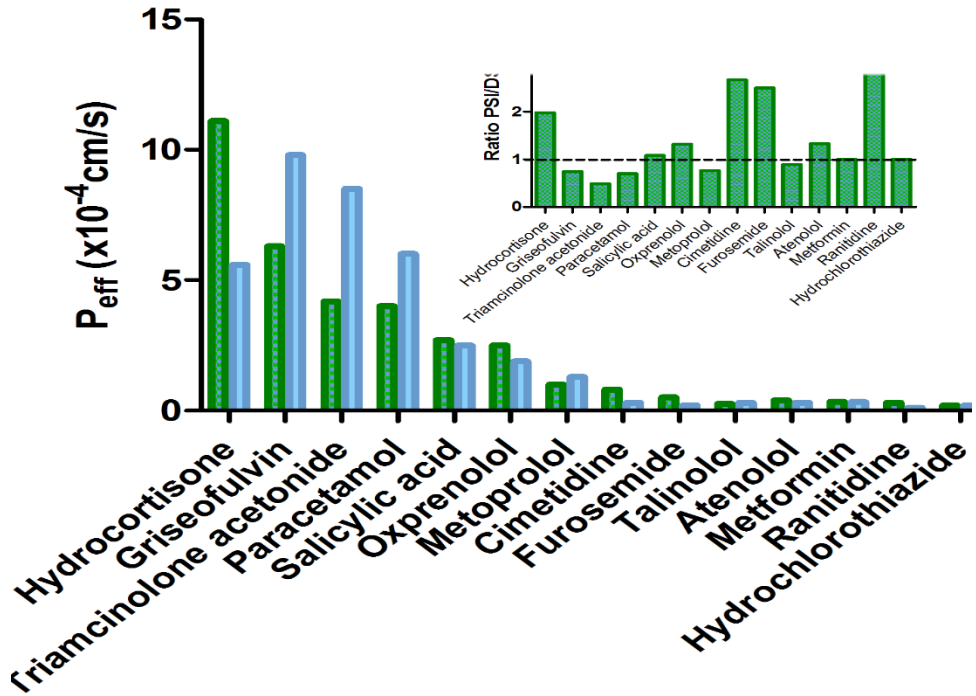

**(Lennernäs. Xenobiotica 1992-2007)**



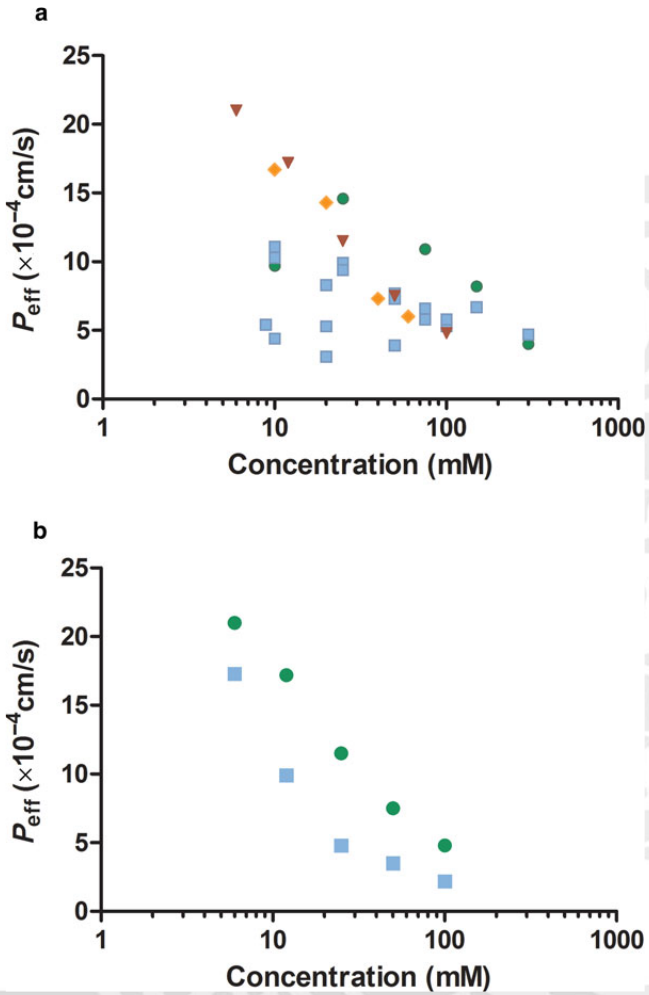



# Proximal (PSI) (green) and distal (DSI) (blue) small intestinal *in vivo* permeation ($P_{eff}$) and regional $P_{eff}$ ratio of 14 model compounds determined using open intestinal perfusion.



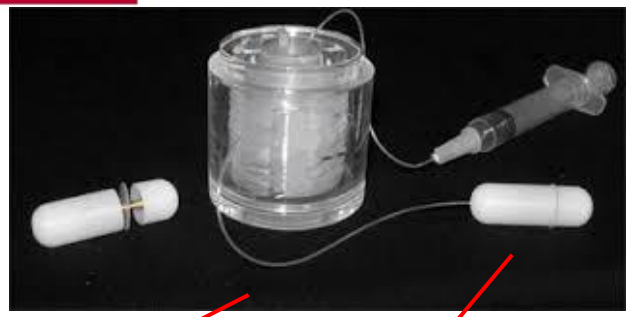

**Schematic presentation (right) of the clinical intestinal perfusion models,**
**A – The open perfusion system,**
**B – The proximal balloon perfusion system,**
**C - The double balloon perfusion system.**




Dahlgren D, Roos C, Sjögren E, Lennernäs H. Direct In Vivo Human Intestinal Permeability (Peff ) Determined with Different Clinical Perfusion and Intubation Methods. J Pharm Sci. 2015 Sep;104(9):2702-26.

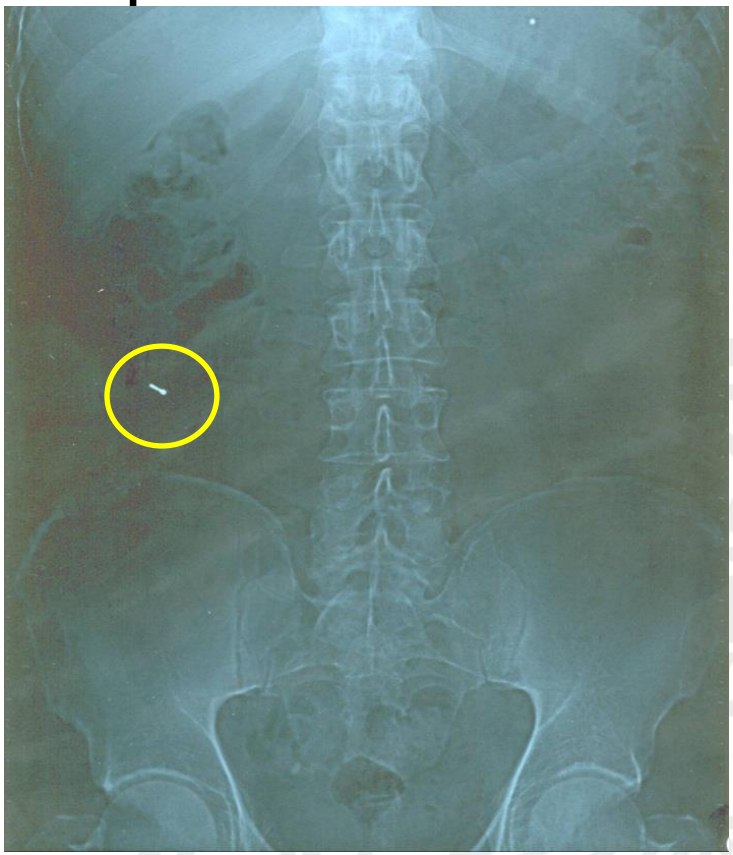
# Human study

## The Bioperm tube-capsule



Diameter
0.6 mm

Size
30 * 10 mm

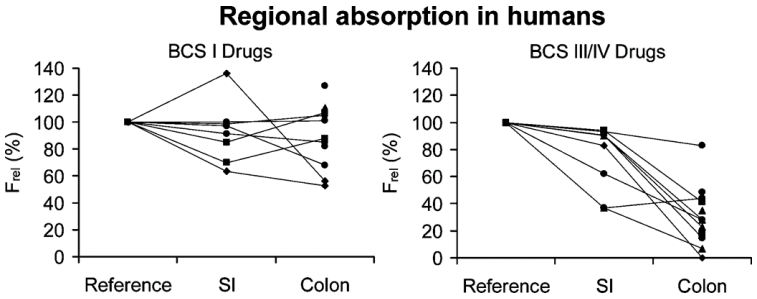


- Capsule positioning verified using:
  - Tube length
    - Jejunum 1 m
    - Ileum 2.5 m
    - Colon 3.5-4 m
  - X-ray (fluoroscopy)


1. **Petri N, Borga O, Nyberg L, Hedeland M, Bondesson U, Lennernas H. Int J Clin Pharmacol Ther. 2006 Feb;44(2):71-9.**
2. **Dahlgren D, Roos C, Lundqvist A, Abrahamsson B, Tannergren C, Hellström PM, Sjögren E, Lennernäs H. Regional Intestinal Permeability of Three Model Drugs in Human. Mol Pharm. 2016 Sep 6;13(9):3013-21.**

# Human study

## Regional intestinal permeability




Tannergren C, Bergendal A, Lennernäs H, Abrahamsson B. Toward an increased understanding of the barriers to colonic drug absorption in humans: implications for early controlled release candidate assessment. Mol Pharm. 2009 Jan-Feb;6(1):60-73.

- Aim
  - To determine reference regional intestinal permeability values of three model drugs in human
- Method
  - Cassette dosing of **atenolol, metoprolol** and **ketoprofen** in solution.
  - 14 volunteers
  - Dosed on 4 occasions
    - 1 x intravenously (1 ml)
    - 3 x intraluminally (13 ml) – jejunum, ileum and colon
  - Blood samples collected following the administrations
  - Individual $P_{eff}$ calculated using the Deconvolution $P_{eff}$ model

molecular pharmaceutics
Article
pubs.acs.org/molecularpharmaceutics

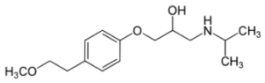
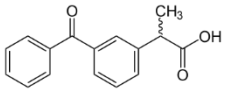
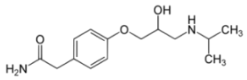
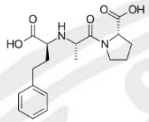
Dahlgren D, Roos C, Sjögren E, Lennernäs H. Direct In Vivo Human Intestinal Permeability (Peff ) Determined with Different Clinical Perfusion and Intubation Methods. J Pharm Sci. 2015 Sep;104(9):2702-26.

**Human *in Vivo* Regional Intestinal Permeability: Quantitation Using Site-Specific Drug Absorption Data**

Erik Sjögren,* David Dahlgren, Carl Roos, and Hans Lennernäs

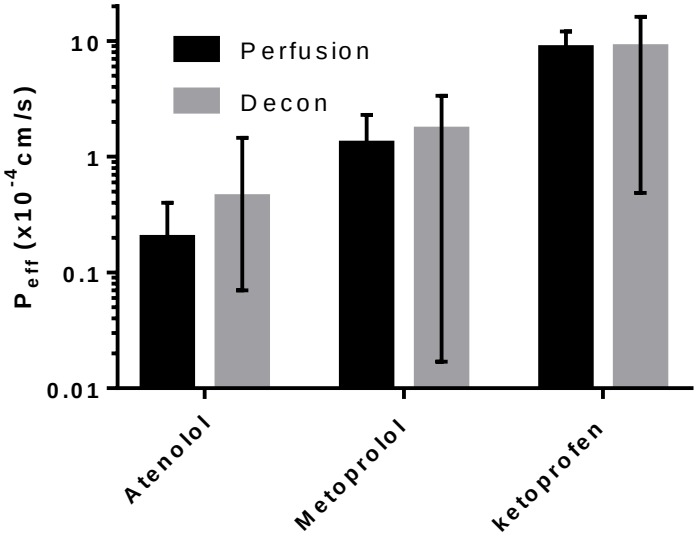
Department of Pharmacy, Biopharmaceutic Research Group, Uppsala University, SE-751 23 Uppsala, Sweden

# Model drugs

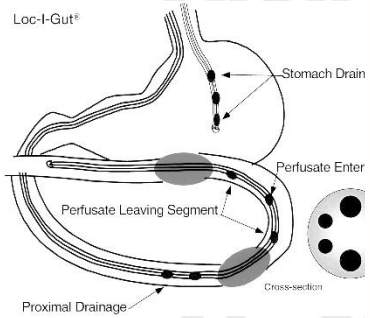
| | Model drugs | | | |
|---|---|---|---|---|
| | Human study | | | |
| | Rat and dog studies | | | |
| | Metoprolol | Ketoprofen | Atenolol | Enalaprilat |
| BCS Class | I | II | III | III |
| Structure |  |  |  |  |
| MW (g/mol) | 267 | 254 | 266 | 348 |
| pKa | 9.6 – base | 3.88 - acid | 9.6 – base | 3.17 - base<br>7.84 - acid |
| Log P | 2.07 | 3.37 | 0.18 | -0.13 |
| Log D pH 7.4 | 0 | 0.1 | -2 | -1.0 |
| Log D pH 6.5 | -0.5 | 0.8 | <-2 | -1.0 |
| PSA | 57.8 | 54.2 | 88.1 | 102.1 |
| HBA/HBD | 4/2 | 3/1 | 4/4 | 6/3 |

# Human study
## Effective jejunal permeability ($P_{eff}$)

| | | Atenolol | Metoprolol | Ketoprofen | |
|---|---|---|---|---|---|
| $P_{eff}$ | **Deconvolution** | **0.45** (0.07-1.46) | **1.72** (0.016-3.38) | **8.85** (0.49-16.14) | median (range) |
| (× $10^{-4}$ cm/s) | **Perfusion** | **0.2** (0.2) | **1.3** (1.0) | **8.7** (3.4) | mean (SD) |

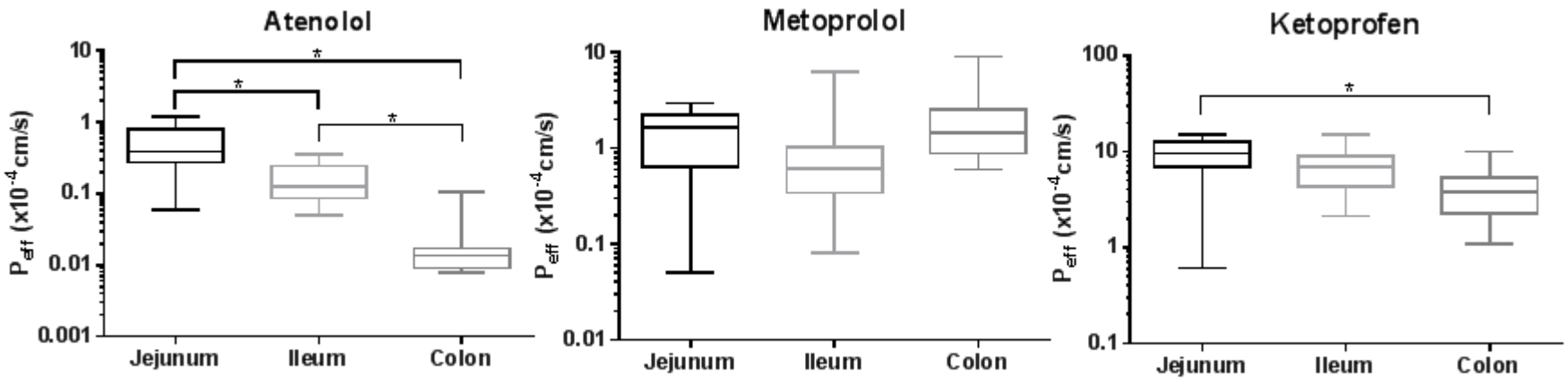



vs.




# Human study

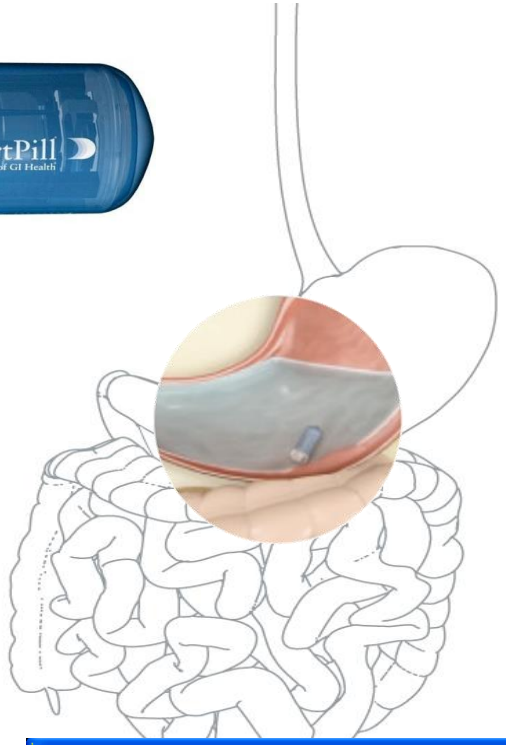
## Regional effective intestinal permeability ($P_{eff}$)




| | | Atenolol | Metoprolol | Ketoprofen |
|---|---|---|---|---|
| $P_{eff}$ (× $10^{-4}$ cm/s) Median (range) | **Jejunum** | **0.45** (0.07-1.46) | **1.72** (0.016-3.38) | **8.85** (0.49-16.14) |
| | **Ileum** | **0.15** (0.06-0.42) | **0.72** (0.09-7.53) | **6.53** (1.49-10.44) |
| | **Colon** | **0.013** (0.01-0.10) | **1.30** (0.54-7.74) | **3.37** (0.98-9.07) |

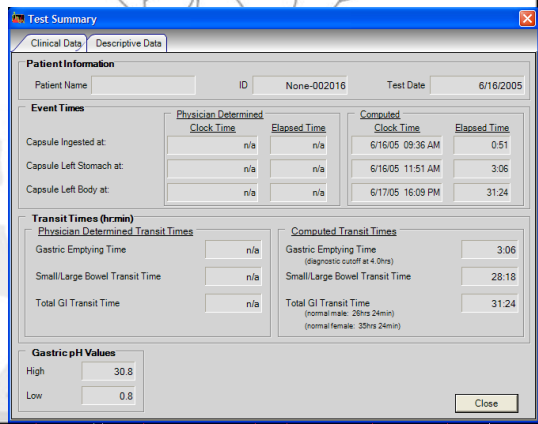
# SmartPill capsule motility procedure and future devices

- Measures pressure, pH and temperature throughout the GI tract.

**These measurements are used to:**

- **Determine gastric emptying time**
- **Determine combined small/large bowel transit time**
- **Intraluminal pH**
- **Determine whole gut transit time**
- **Characterize pressure patterns and provide motility indices for the antrum and duodenum**
- **To dose and calculate regional intestinal permeability/absorption (depend on availability of IV dosing)**



Test Summary

Clinical Data | Descriptive Data

**Patient Information**

| Patient Name | | ID | None-002016 | Test Date | 6/16/2005 |
|---|---|---|---|---|---|

**Event Times**

| | Physician Determined Clock Time | Physician Determined Elapsed Time | Computed Clock Time | Computed Elapsed Time |
|---|---|---|---|---|
| Capsule Ingested at: | n/a | n/a | 6/16/05 09:36 AM | 0:51 |
| Capsule Left Stomach at: | n/a | n/a | 6/16/05 11:51 AM | 3:06 |
| Capsule Left Body at: | n/a | n/a | 6/17/05 16:09 PM | 31:24 |

**Transit Times (hr:min)**

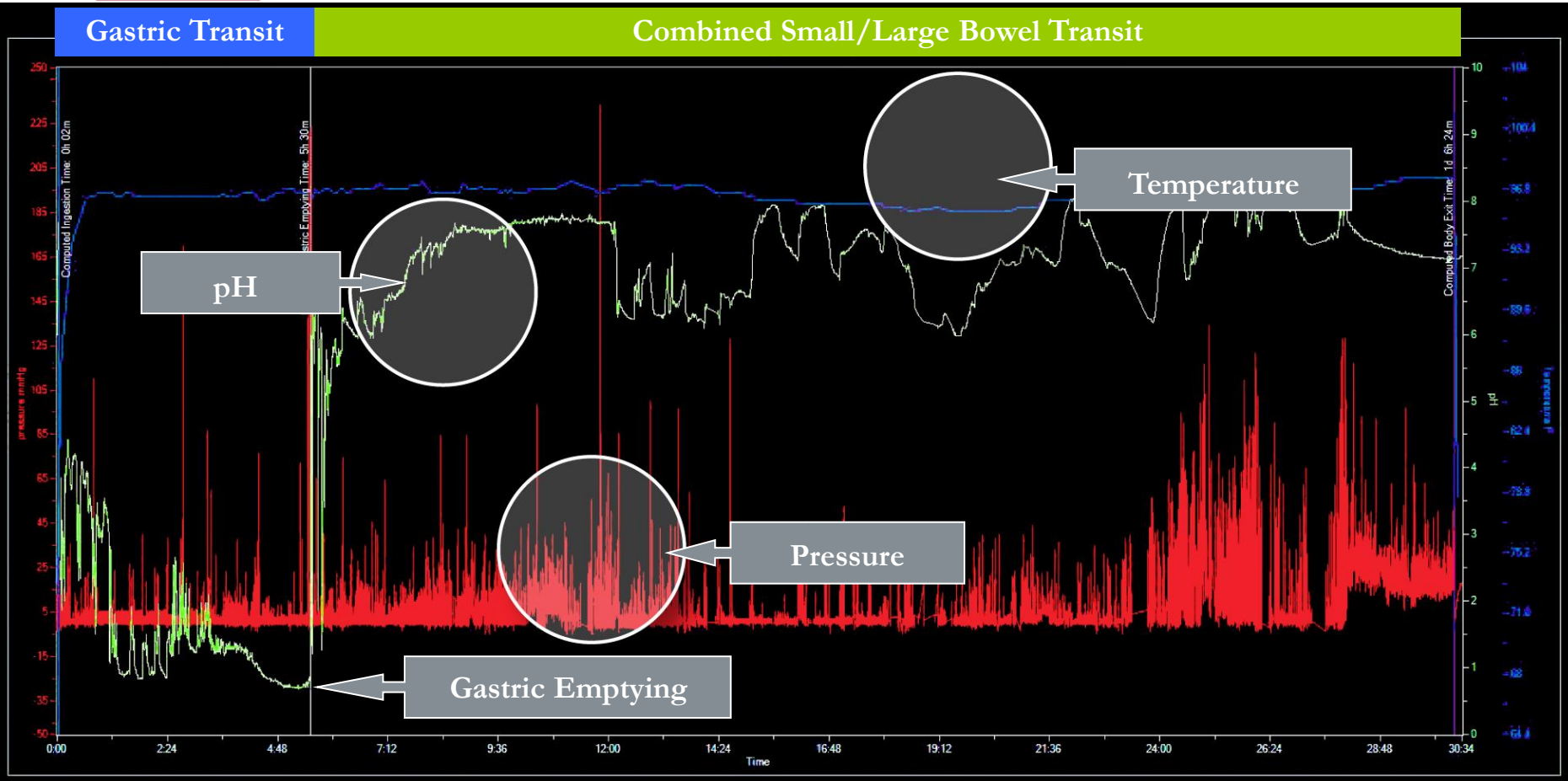
| Physician Determined Transit Times | | Computed Transit Times | |
|---|---|---|---|
| Gastric Emptying Time | n/a | Gastric Emptying Time (diagnostic cutoff at 4.0hrs) | 3:06 |
| Small/Large Bowel Transit Time | n/a | Small/Large Bowel Transit Time | 28:18 |
| Total GI Transit Time | n/a | Total GI Transit Time (normal male: 26hrs 24min) (normal female: 35hrs 24min) | 31:24 |

**Gastric pH Values**

| | |
|---|---|
| High | 30.8 |
| Low | 0.8 |

Close

# SmartPill – GI-transit, motility, intraluminal pressure and pH



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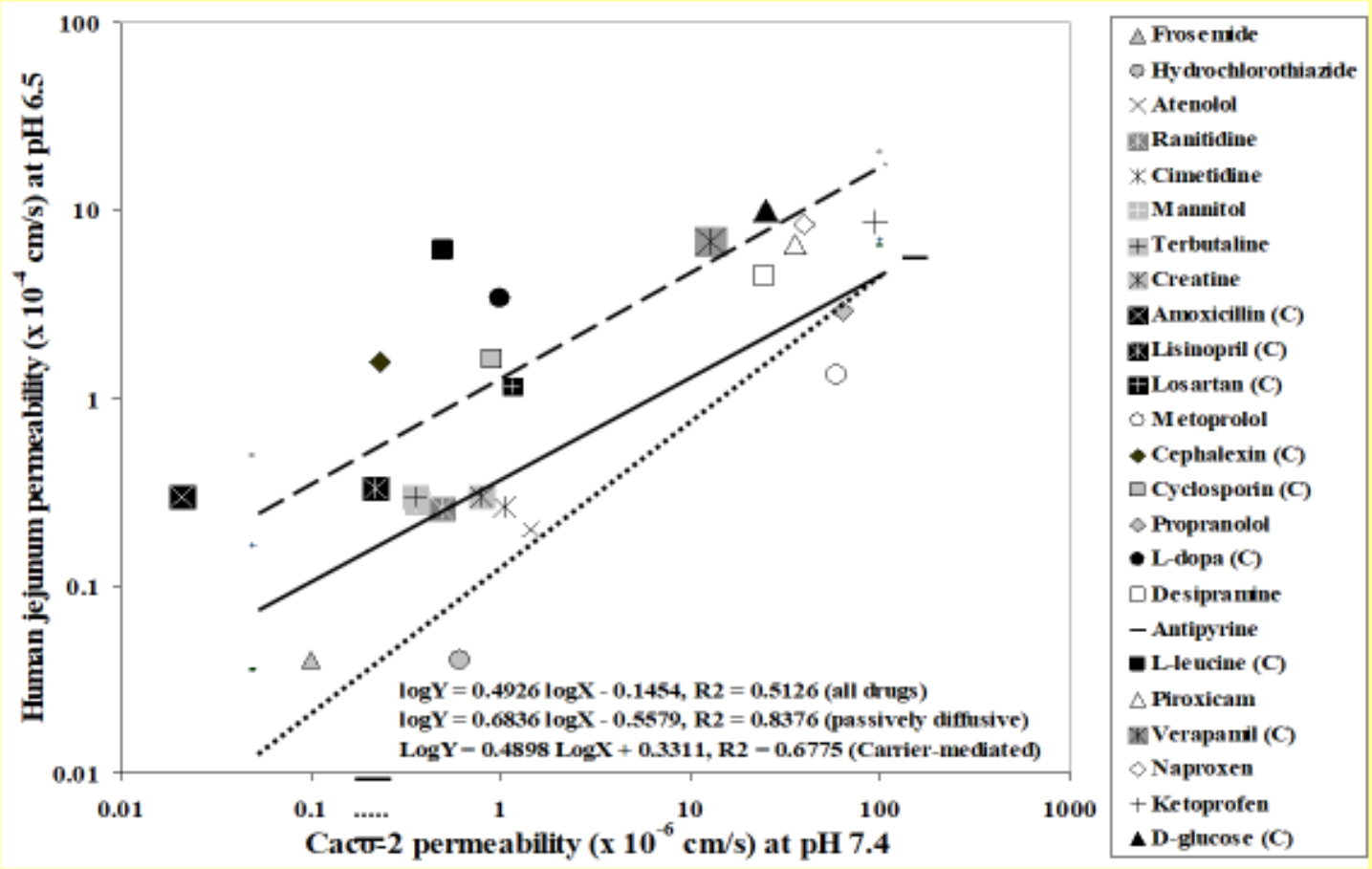
# Human jejunal Permebeility vs human colorectal adenocarcinoma (Caco-2) first isolated in 1977 from a 72-year-old Caucasian male)




Sun, D.; Lennernas, H.; Welage, L. S.; Barnett, J.; Landowaki, C. P. et al. A Comparison of Human and Caco 2 Gene Expression Profiles for 12,000 Genes and the Permeabilities of 26 Drugs in the Human Intestine and Caco 2 Cells. *Pharm Res* 2002, *19*, 1398–1413

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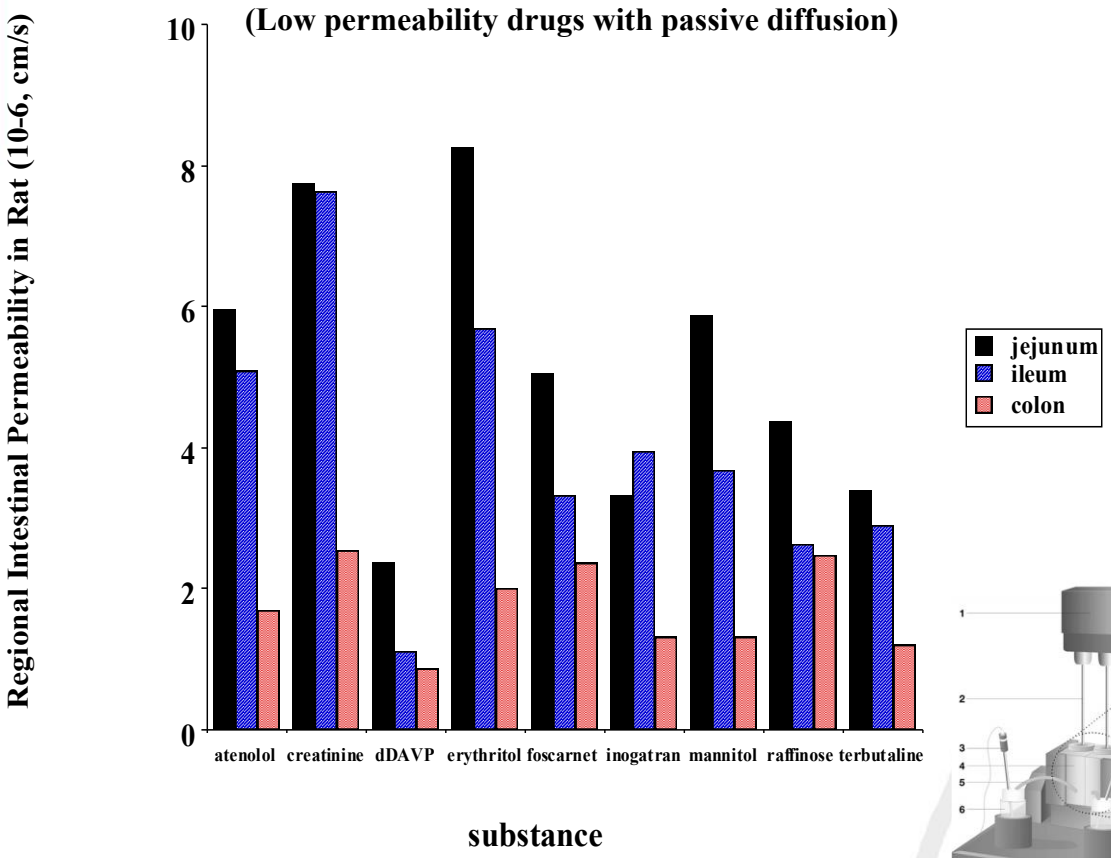
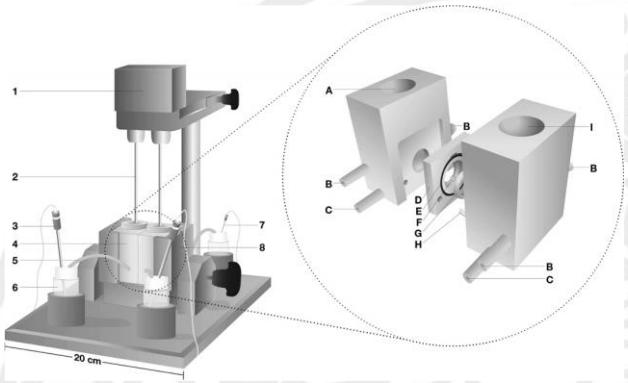
**Apparent permeabilities in various intestinal regions in rats.**




**Cholesterol, sphingolipids, and glycolipids, constituents in the cell membrane that modulate its properties, and subsequently the permeability to drus. In particular, membranes containing cholesterol exhibit greater acyl-chain order and increased stiffness, reflected in a smaller average area per lipid, and, hence, a reduced permeability to substrates.**

Ungell A-L, Nylander S., Bergstrand S., Sjöberg Å and Lennernäs H. Membrane transport of drugs in different regions of the intestinal tract of the rat. J. Pharm. Sci. 87, 360-366, (1998).

Sjöberg Å, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell AL. Comprehensive study on regional **human** intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. Eur J Pharm Sci. 2013 Jan 23;48(1-2):166-80.



**Table 2**
Apparent permeability ($P_{app}$) for compounds in human intestinal tissue in the modified Ussing chamber. Results are presented as mean ± SD ($n = x$). Numbers indicate the compounds used in the jejunum correlation curve and letters indicate the compounds for colonic correlation curve.

| | | | | Apparent permeability, $P_{app}$ [$\times 10^{-6}$ cm/s] (mucosa to serosa direction) | | | |
|---|---|---|---|---|---|---|---|
| Comp. No. | Compound | Fa (%) p.o* | Fa (%) i.c.** | Duodenum | Jejunum | Ileum | Colon |
| 1 | Sulphasalazine | 12 | | | 0.09 ± 0.06 (2) | | |
| A | Fexofenadine | | 13 | | | | 0.22 ± 0.08 (4) |
| B | Ranitidine | | 9 | | | | 0.62 ± 0.29 (4) |
| 2 | Vinblastine | 25 | | | 1.31 ± 2.14 (4) | | |
| 3,C | Digoxin | 81 | 66 | | 1.44 ± 0.72 (7) | | 2.83 ± 1.26 (4) |
| 4,D | Melagatran | 6 | 3 | | 1.51 ± 0.39 (3) | | 1.20 ± 1.07 (2) |
| 5,E | Ximelagatran | 45 | 39 | 3.15 ± 0.07 (2) | 3.03 ± 1.08 (5) | 1.01 (1) | 1.90 ± 0.66 (4) |
| 6 | Quinidine | 80 | | | 3.36 ± 0.56 (5) | | |
| 7 | Cimetidine | 64 | | | 3.74 ± 0.47 (5) | | |
| 8,F | Atenolol | 56 | 28 | 2.61 (1) | 4.11 ± 1.32 (4) | | 1.27 ± 0.64 (5) |
| 9 | Oseltamivir | 81 | | | 4.64 ± 1.17 (2) | | |
| 10 | Mannitol | 38 | | 4.85 ± 0.86 (5) | 5.56 ± 2.01 (253) | 1.39 ± 0.56 (3) | 1.22 ± 0.79 (104) |
| 11 | Rosuvastatin | 50 | | | 6.95 ± 1.05 (3) | | 1.07 ± 0.25 (2) |
| 12 | Diclofenac | 99 | | | 8.55 ± 0.19 (2) | | |
| 13,G | Oxprenolol | 90 | 74 | | 9.50 ± 1.73 (2) | | 30.4 ± 8.6 (4) |
| 14,H | Metoprolol | 95 | 100 | | 15.9 ± 3.69 (4) | | 18.8 ± 4.00 (3) |
| 15 | Creatinine | 100 | | 4.24 (1) | 20.2 ± 7.84 (4) | | 2.55 ± 2.74 (3) |
| 16 | Hydrocortisone | 89 | | 13.5 ± 5.45 (3) | 22.3 ± 4.22 (4) | | 15.0 ± 6.49 (4) |
| I | Diltiazem | | 82 | | | | 23.6 ± 2.3 (3) |
| 17 | Propranolol | 95 | | 18.0 ± 6.72 (4) | 31.9 ± 17.0 (24) | 22.4 ± 14.6 (5) | 35.4 ± 14.2 (7) |
| 18 | Verapamil | 100 | | | 36.0 ± 10.0 (4) | | |
| 19 | Midazolam | 100 | | | 38.0 ± 16.0 (2) | | |
| 20,J | Salicylic acid | 100 | 100 | 18.4 (1) | 41.1 ± 16.5 (4) | | 13.3 ± 3.11 (2) |
| 21 | Indomethacine | 100 | | | 48.2 ± 12.4 (4) | | |
| 22 | Antipyrine | 100 | | 26.3 ± 6.99 (3) | 49.7 ± 13.3 (4) | | 54.6 ± 13.0 (3) |
| 23 | Testosterone | 100 | | | 68.0 ± 10.9 (6) | | |
| 24 | D-Glucose | 100 | | | 113 ± 49.4 (29) | | 1.02 ± 0.52 (4) |
| 25 | L-Leucine | 100 | | | 140 ± 40.8 (4) | | 2.63 ± 0.86 (3) |

$P_{app}$ for some of the compounds has been presented in the paper by Ungell (2002).
* Fraction absorbed data obtained from references: Galetin and Houston, 2006; Tannergren et al., 2009; Thelen et al., 2011; Ungell, 2002; Ungell and Karlsson, 2003. In house data for ximelagatran and rosuvastatin.
** Fraction absorbed data obtained from reference: Tannergren et al., 2009.

# Experimental Determination of the Membrane Permeability in different types of assays to provide estimates of the apparent membrane permeability

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Chipot C. Predictions from First-Principles of Membrane Permeability to Small Molecules: How Useful Are They in Practice? J Chem Inf Model. 2023 Aug 14;63(15):4533-4544.

pubs.acs.org/JCIM Perspective

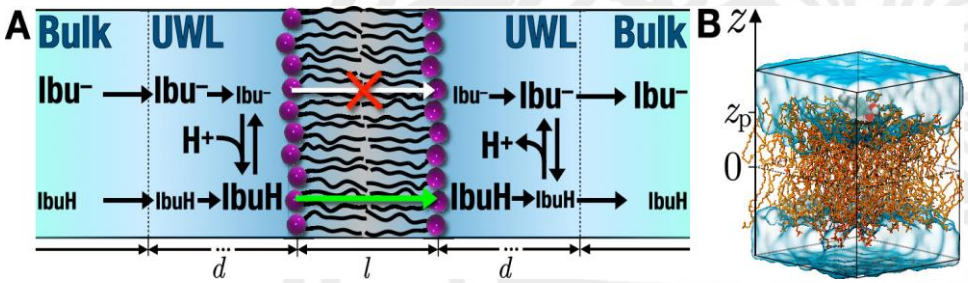
**Table 1. Apparent Membrane Permeability ($P_{app}$) of Ibuprofen Measured Using a Variety of Methods, from In Vitro Artificial Membrane Assays to In Vivo Animal Intestinal Perfusion**

| Assay | $P_{app}$ (cm/s) | reference |
|---|---|---|
| Caco-2 | $52.5 \times 10^{-6}$ | |
| PAMPA (pH 5.5) | $10.8 \times 10^{-6}$ | 21 |
| PAMPA (pH 7.4) | $6.8 \times 10^{-6}$ | |
| Caco-2 | $30.1 \times 10^{-6}$ | 22 |
| rat intestinal | $200 \times 10^{-6}$ | 23 |
| solution-based PAMPA | $10.8 \times 10^{-6}$ | |
| trilayer PAMPA | $6.8 \times 10^{-6}$ | 24 |
| phospholipid vesicle (pH 6.2) | $13.3 \times 10^{-6}$ | |
| phospholipid vesicle (pH 7.4) | $8.4 \times 10^{-6}$ | 25 |
| vinblastine-treated Caco-2 | $76.2 \times 10^{-6}$ | |
| MDCK-MDR1[a] | $74.3 \times 10^{-6}$ | 26 |
| jejunum | $228 \times 10^{-6}$ | |
| middle small intestine | $205 \times 10^{-6}$ | 27 |
| ileum | $206 \times 10^{-6}$ | |
| Permeapad[28] | $16.6 \times 10^{-6}$ | 29 |

[a]MDCK cells with the MDR1 gene encoding for efflux P-glycoprotein.

The apparent membrane permeability, Papp, depends on the nature of the cell-line assay, and applied conditions of the experiment, and will vary from batch to batch.

Which membrane model would provide the most accurate and precise representation of the permeability assays. How can these values be used theoretical of Pm in drug discovery as well as PBBM.




How useful are membrane-permeability estimates from computer based molecular dynamics simulations by using the inhomogeneous solubility–diffusion model ?

The gastrointestinal tract plays a central role in the maintenance of homeostasis in the body through endocrine and immune functions as well as digestive functions. Gastrointestinal-released hormones and an interplay with neurosignaling play a significant role in these processes.

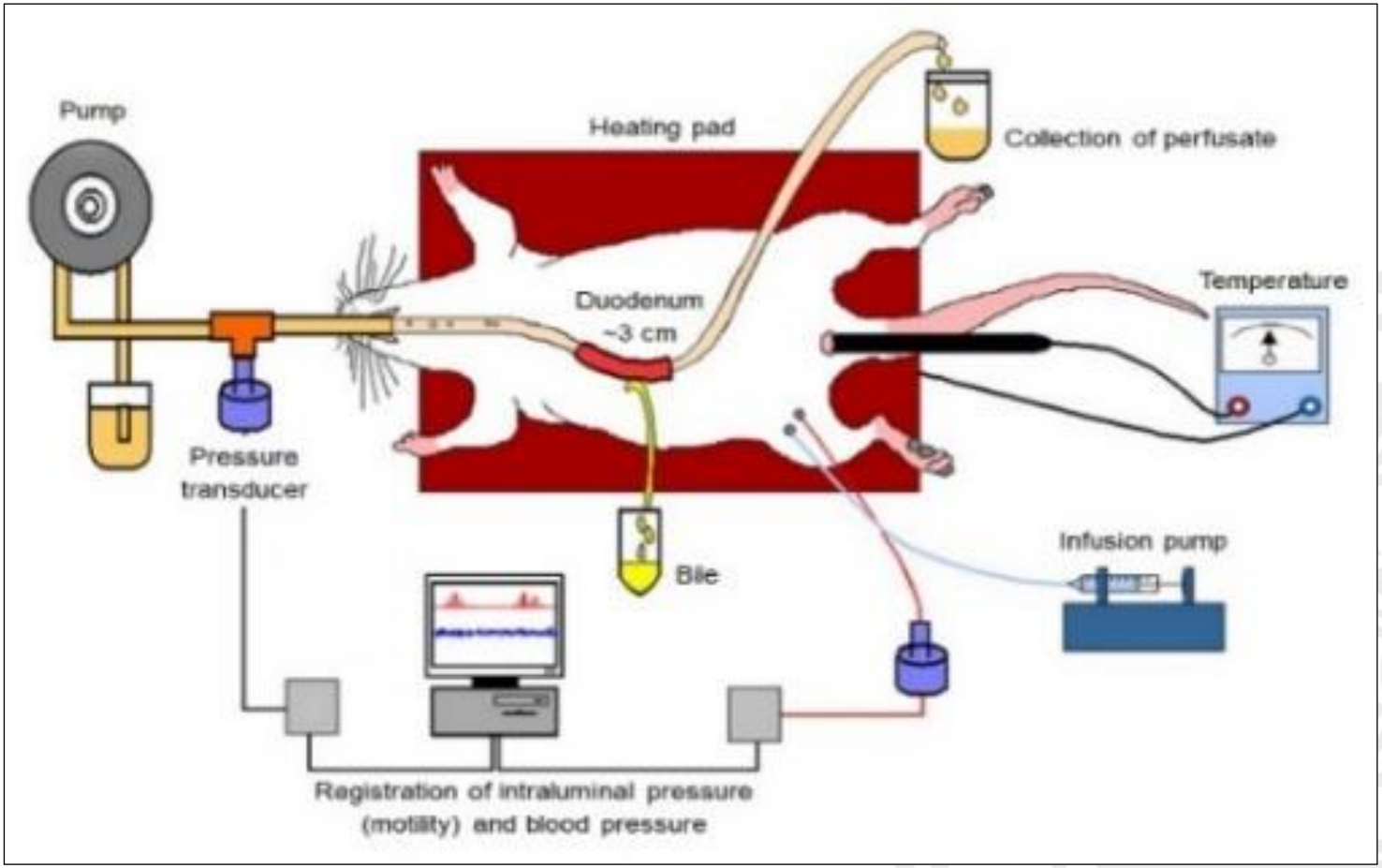
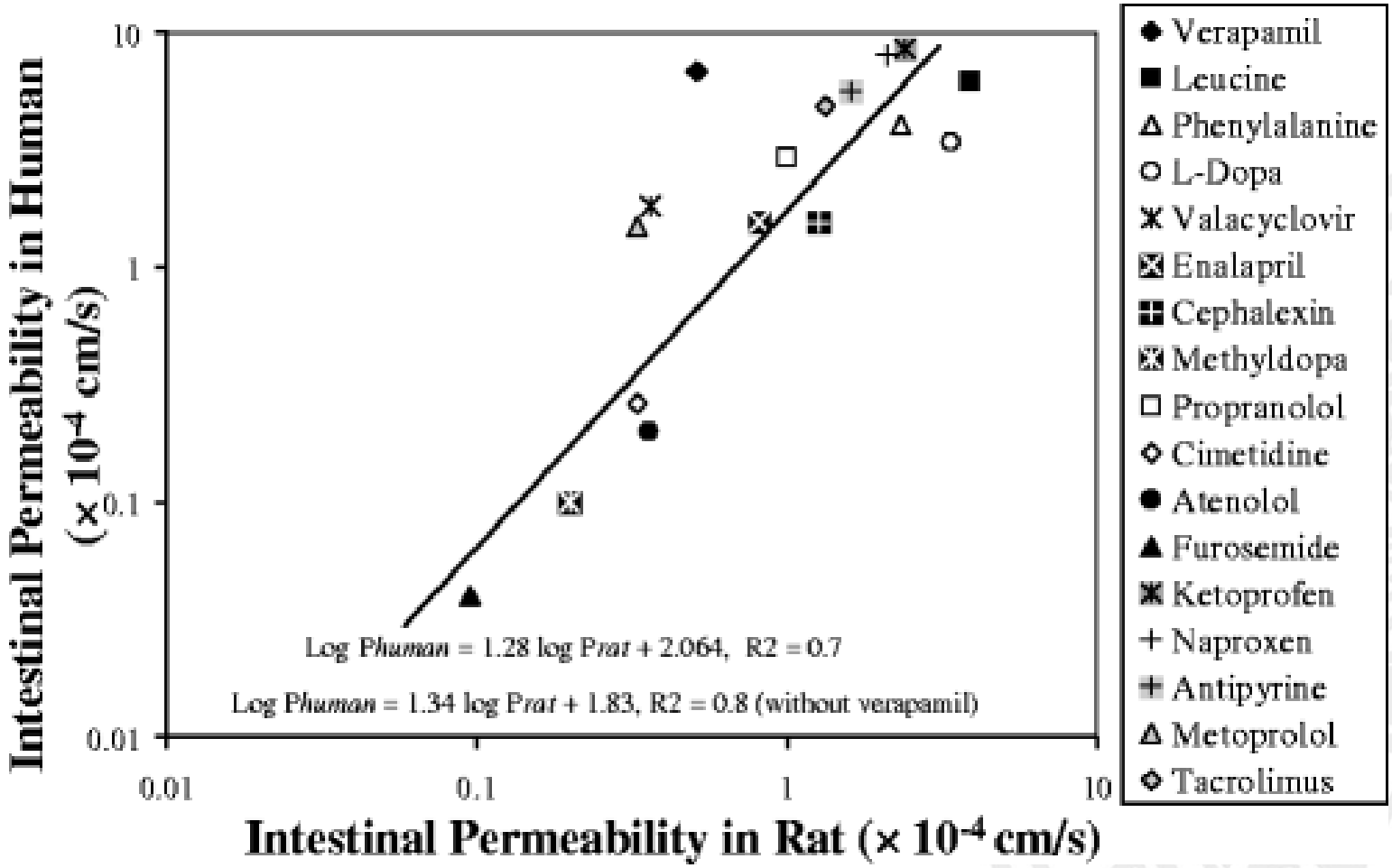



**Figure 2. Schematic illustration of the in vivo rat single-pass intestinal perfusion (SPIP) model, allowing continuous monitoring intestinal epithelial permeability, metabolism, motility, ion transport, fluid flux and systemic blood pressure.**

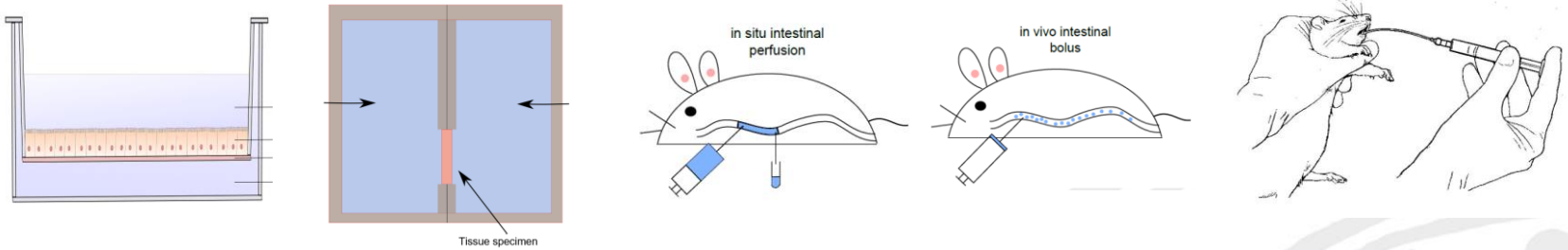
# Correlation of oral permeability between rat and human

Why is it Challenging to Predict Intestinal Drug Absorption
and Oral Bioavailability in Human Using Rat Model
Xianhua Cao, Seth T. Gibbs, Lanyan Fang, Heather A. Miller,
Christopher P. Landowski, Ho-Chul Shin, Hans Lennernas,
Yanqiang Zhong, Gordon L. Amidon, Lawrence X. Yu and Duxin Sun
Pharm Res. 2006 23(8):1675-86.

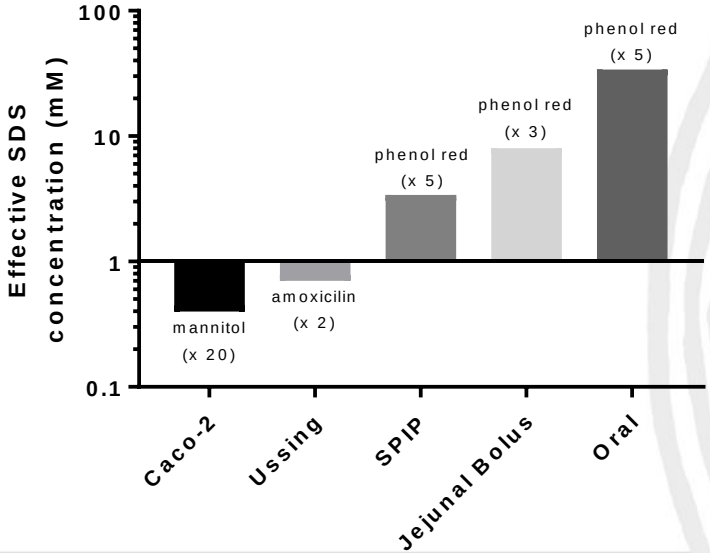


# The model is important! (effect of excipients)

**In vivo likeness of preclinical models**

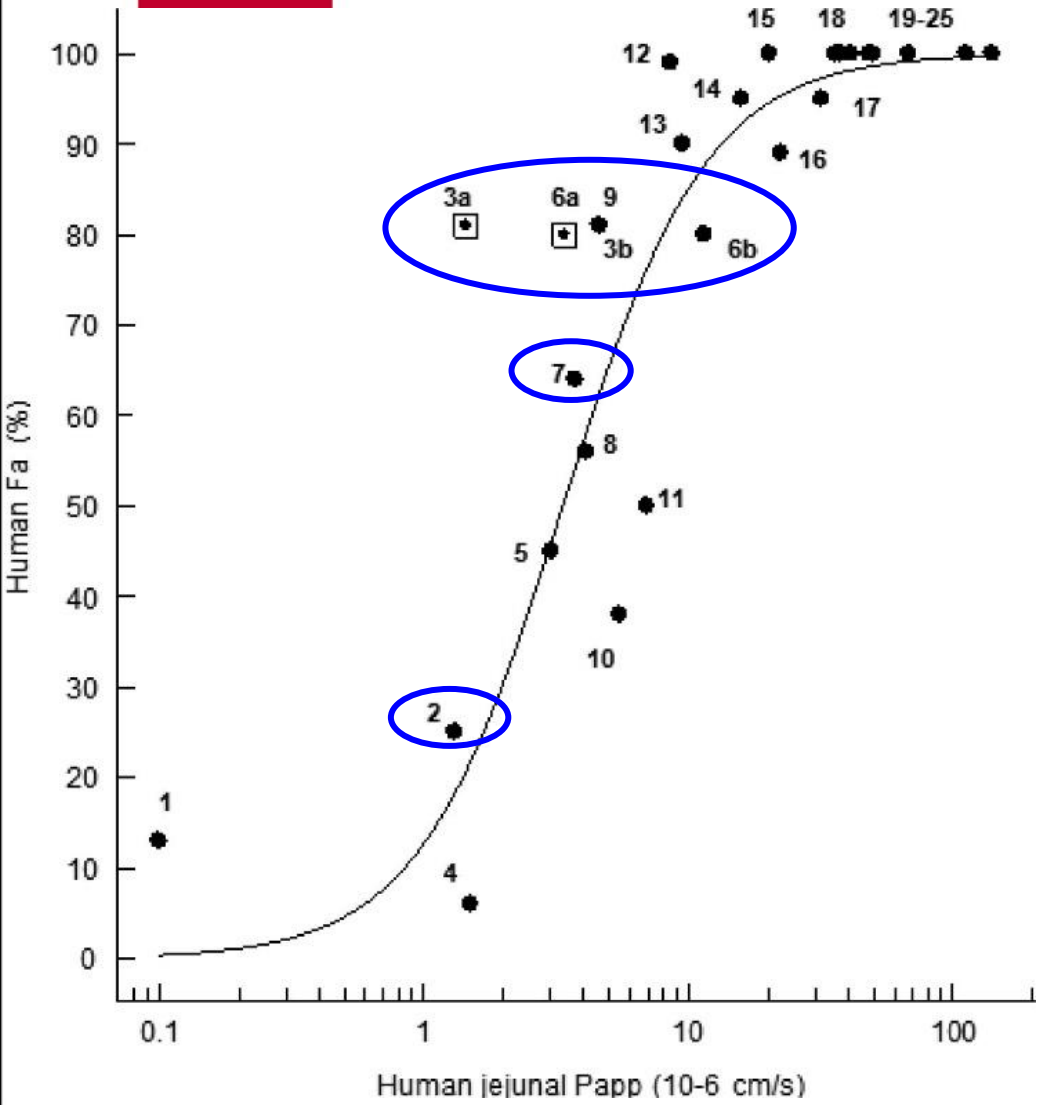



Caco-2 | Rat Ussing | Rat SPIP | Rat jejunum | Rat oral




Dahlgren D, Lennernäs H. Intestinal Permeability and Drug Absorption: Predictive Experimental, Computational and In Vivo Approaches. Pharmaceutics. 2019 Aug 13;11(8):411.

# Bidirectional permeability to assess uptake or **efflux**




Efflux ratio in this study for vinblastine (2), digoxin (3a,3b), cimetidine (7) and quinidine (6a,6b) were ER = 4.25, 5.41, 1.79 and 5.85

Sjöberg Å, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell AL. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. Eur J Pharm Sci. 2013 Jan 23;48(1-2):166-80.

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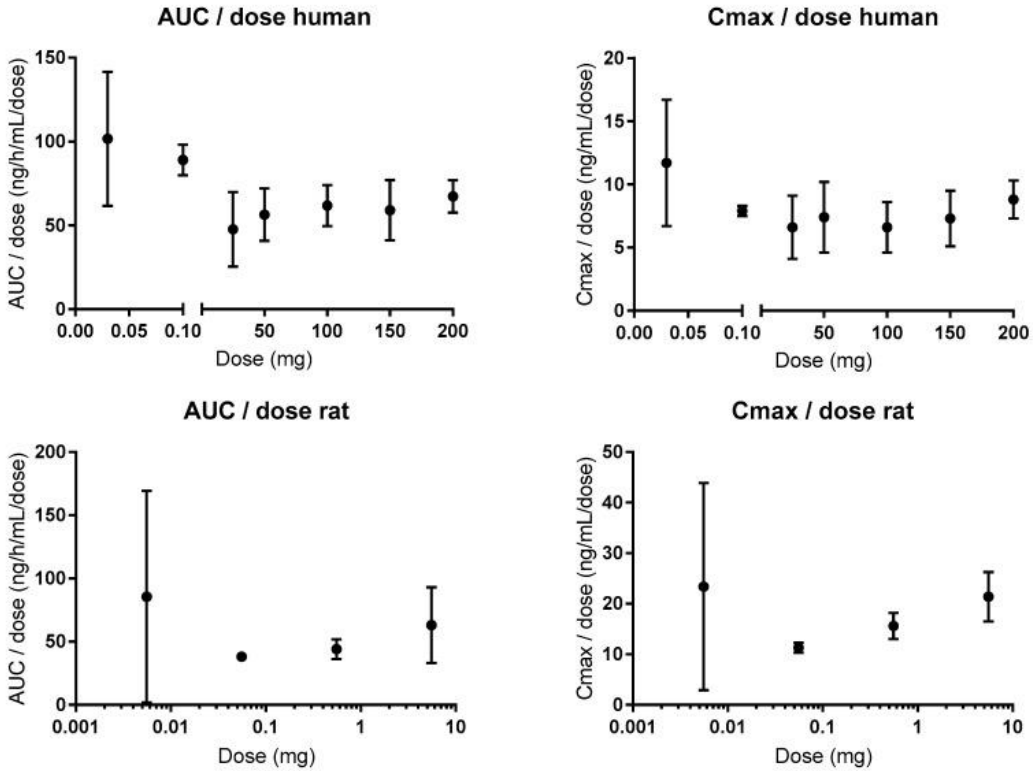
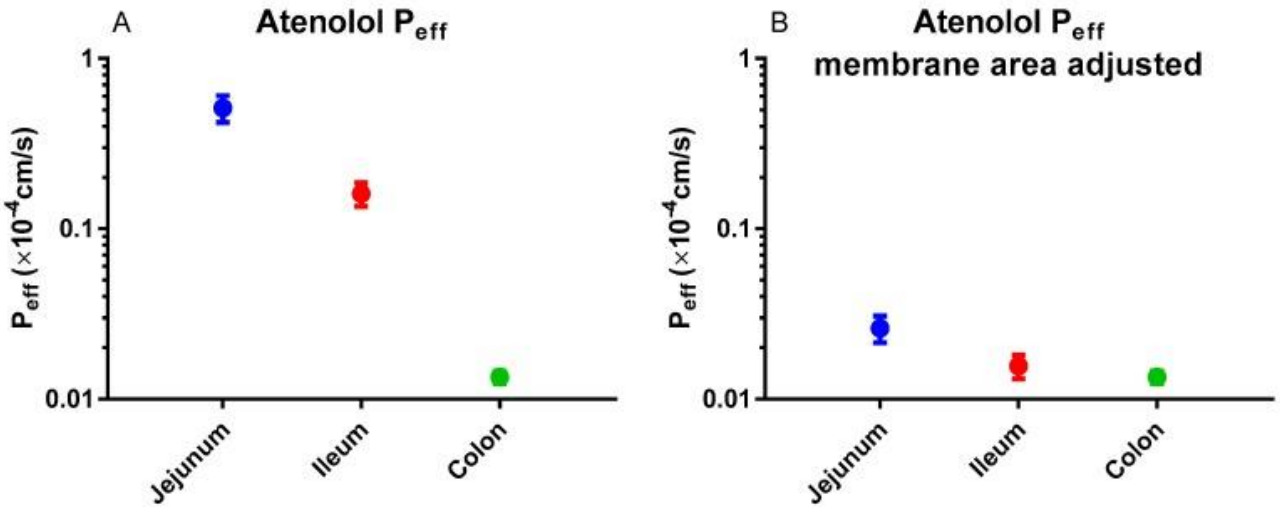
Dahlgren D, Lennernäs H. Intestinal Permeability and Drug Absorption: Predictive Experimental, Computational and In Vivo Approaches. Pharmaceutics. 2019 Aug 13;11(8):411.



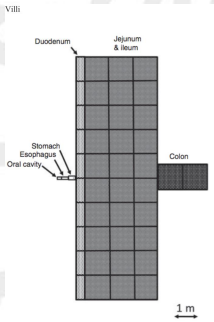

**Dose proportionality in the AUC and Cmax of atenolol in humans (0.1–200 mg) and rats (0.55 µg–5.5 mg).**

**Efflux ratio for atenolol in Caco-2 model range from 0.18-3.8**




**(A) Regional intestinal effective permeability (Peff) of atenolol in humans. (B) Surface area (villi and folds)-adjusted regional intestinal Peff values for atenolol in humans: jejunum 19-fold, ileum 10-fold, colon 1-fold**

A B




Colonic membranes containing cholesterol exhibit greater acyl-chain order and increased stiffness, and hence a reduced permeability to substrates.

# Trends in Molecular Properties, Bioavailability, and Permeability across the Bayer Compound Collection (O'Donovan et al.)

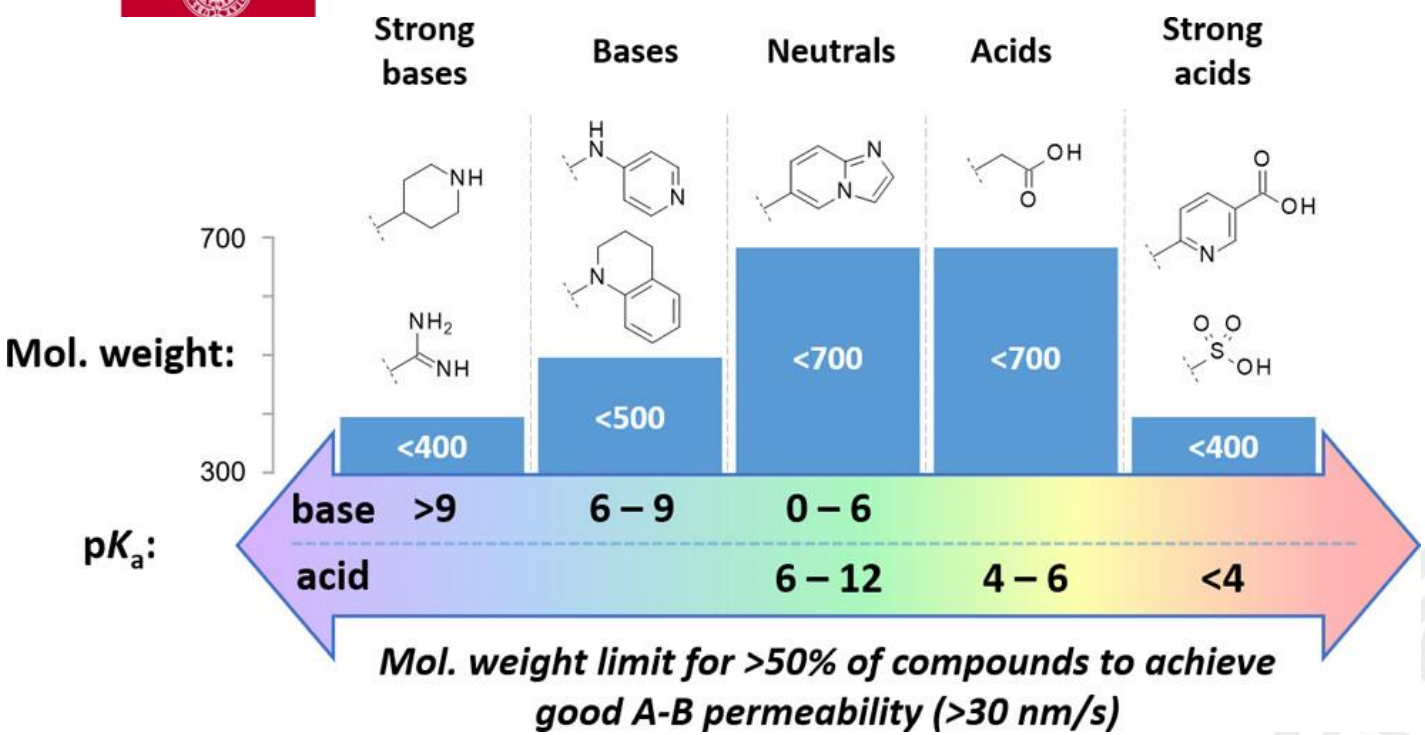
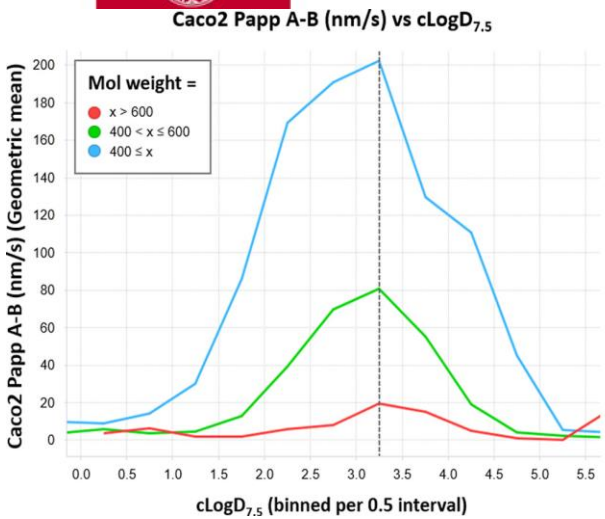


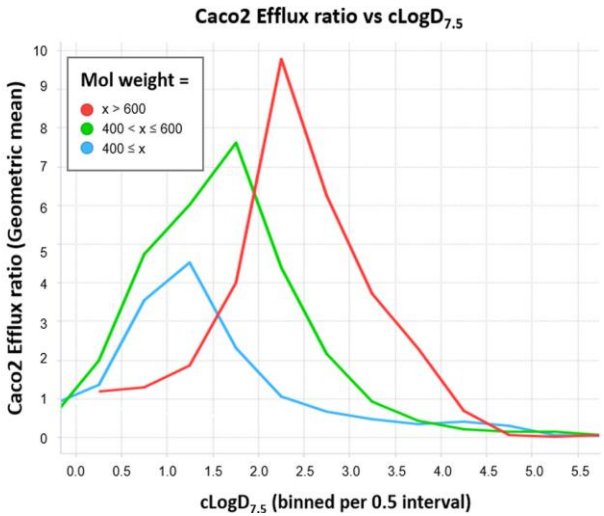

Figure 8. Molecular weight and pKa ranges at which >50% of compounds exhibit moderate to good permeability in the Caco2 Papp A-B assay (>30 nm/s). pKa values are stated for the most basic and the most acidic centers in a compound.

*J. Med. Chem.* 2023, 66, 4, 2347−2360

DOI: (10.1021/acs.jmedchem.2c01577)

# Trends in Molecular Properties, Bioavailability, and Permeability across the Bayer Compound Collection (O'Donovan et al.)

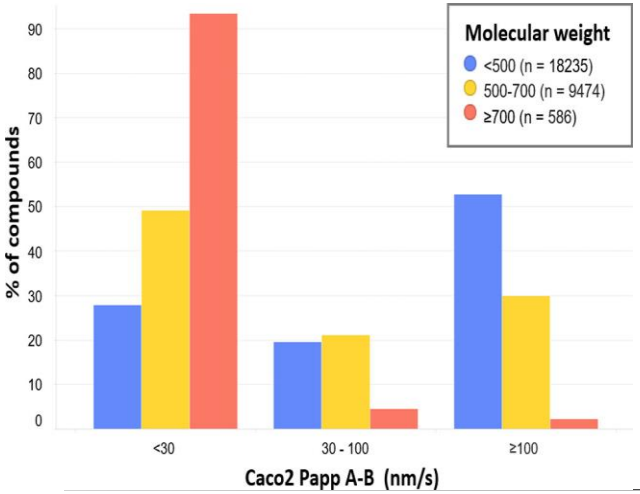



(*Left*) Relationship between cLogD7.5 and geometric mean of Caco2 Papp A-B (nm/s), binned per 0.5 interval of cLogD7.5.

Lines are colored by molecular weight range (blue = ≤400, green = 400–600, red = >600). Extreme values have been omitted as outliers

(*Right*) Relationship between cLogD7.5 and geometric mean of Caco2 efflux ratio, binned and colored as per left-hand figure




Bar chart showing the distribution of Caco2 apparent permeability A-B (nm/s) values for compounds with Ro5-like (<500), eRo5-like (500–700), and bRo5-like (≥700) molecular weights.

Interesting challenges for novel drug candidate beyond Lipinski rule of 5!

**Recognized difficulties for *in vitro* permeability assessments for these larger and lipophilic drug candidates!**

*J. Med. Chem.* 2023, 66, 4, 2347−2360

DOI: (10.1021/acs.jmedchem.2c01577)

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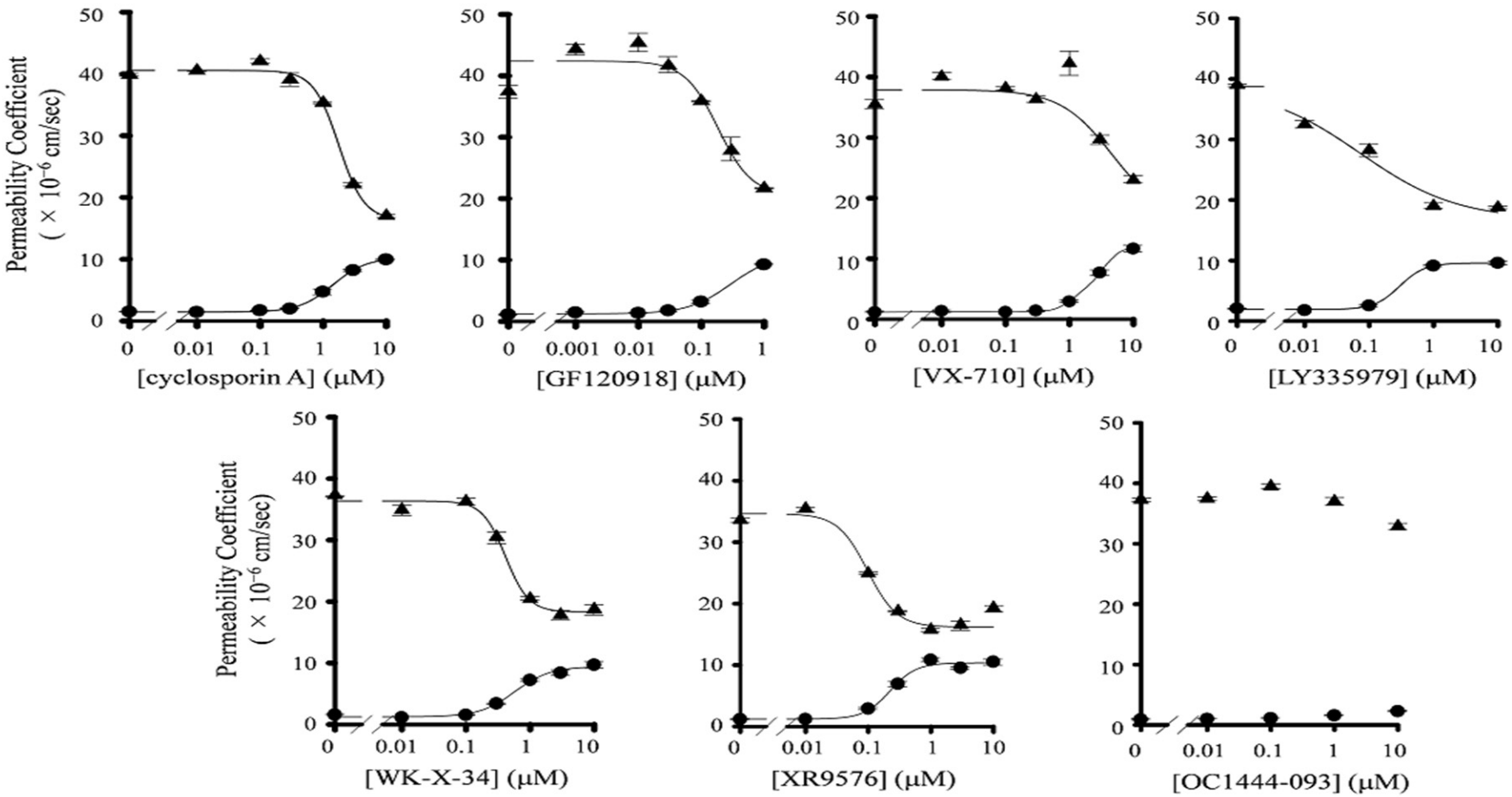
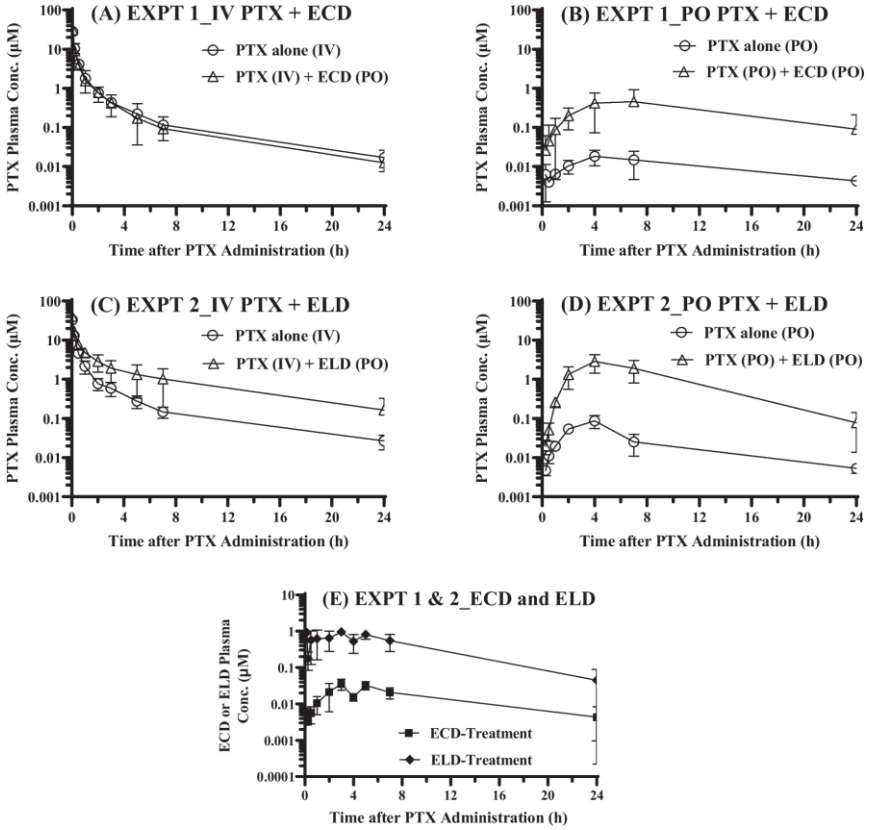
# In vivo role of intestinal efflux

Inhibitory effect of P-gp inhibitors on P-gp-mediated efflux of paclitaxel in Caco-2 cells. The apical-to-basal (AP-to-BL) (●), and basal-to-apical (BL-to-AP) (▲).



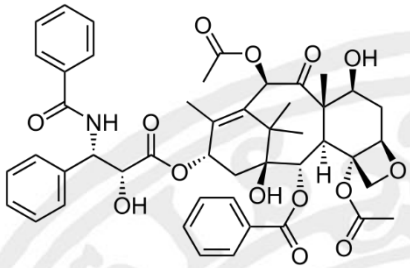

Kono Y, Kawahara I, Shinozaki K, Nomura I, Marutani H, Yamamoto A, Fujita T. Characterization of P-Glycoprotein Inhibitors for Evaluating the Effect of P-Glycoprotein on the Intestinal Absorption of Drugs. Pharmaceutics. 2021 Mar 15;13(3):388.

# Evaluation of Encequidar (ECD) and Elacridar (ELD) as An Intestinal P-gp and BCRP Specific Inhibitors to Assess the Role of Intestinal P-gp and BCRP in Drug-Drug Interactions with paxlitaxel (PTX)




Chu J, Panfen E, Wang L, Marino A, Chen XQ, Fancher RM, Landage R, Patil O, Desai SD, Shah D, Xue Y, Sinz M, Shen H.

Pharm Res. 2023

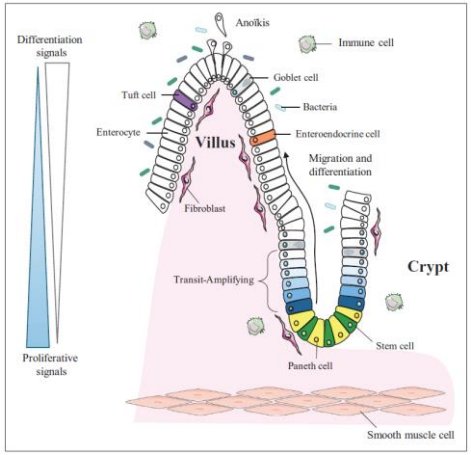


| | |
|---|---|
| Molar mass | 853.91 g/mol |
| Log P | 3.2-26 |
| Hydrogen Acceptor Count | 10 |
| Hydrogen Donor Count | 4 |
| Physiological charge | 0 |
| Polar Surface Area | 221.29 Å² |

Mean plasma concentration–time profile of PTX in rats after a single intravenous (IV; 5 mg/kg) or oral dose (PO; 20 mg/kg) of PTX alone (open circles) and following a single dose of PTX with oral ECD (A and B) or ELD (C and D) (open triangles)

# In vitro models of intestinal epithelium: Toward bioengineered systems

Creff J et al Journal of Tissue Engineering
Volume 12: 1–16



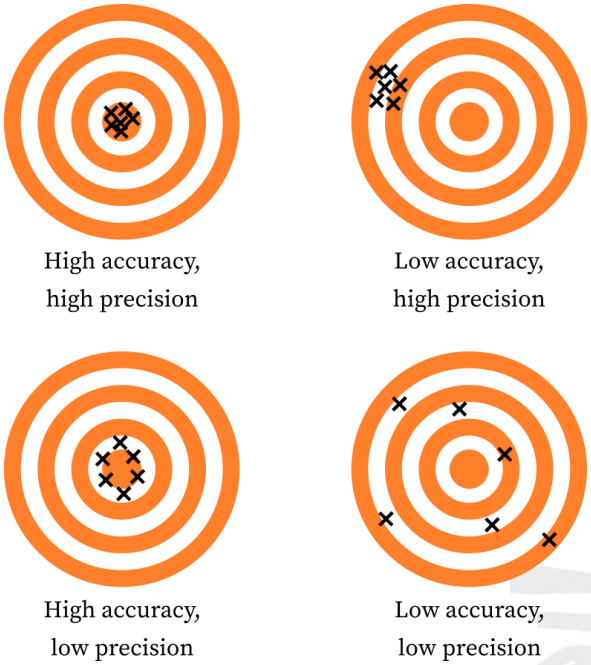




**Table 1.** Sources of cells to study intestinal physiology.

| Cell source | Culture model | Advantages | Disadvantages | Applications |
|---|---|---|---|---|
| Adult stem cells<br>Dissociated fresh crypt<br>Dissociated organoids | Organoids<br>3D scaffolds<br>Gut-on-chip<br>2D monolayer | Human or murine origin<br>Heterogeneity | Adult stem cells<br>Only epithelial cells | Genetic disease<br>Drug screening<br>Host/pathogen interaction<br>Adult epithelial function<br>Adult intestinal stem cell biology<br>Tissue engineering |
| Pluripotent stem cells (iPS) | Organoids<br>Gut-on-chip | Human origin<br>Presence of mesenchyme<br>Recapitulate the development | Immature features require maturation in vivo or *in vitro* | Genetic disease<br>Drug screening<br>Host/pathogen interaction<br>Organ development<br>Tissue interaction<br>Tissue engineering |
| Cancer cell lines<br>Caco-2<br>Caco-2/HT29<br>SW480 | 2D monolayer<br>transwell<br>3D scaffolds<br>Gut-on-chip | Human origin<br>Easy and more affordable | Lack of heterogeneity<br>Cancer origine | Cellular and molecular biology<br>Absorption and toxicology assays<br>Host/pathogen interaction<br>Tissue engineering |

# Future in vitro intestinal peremabeility models?

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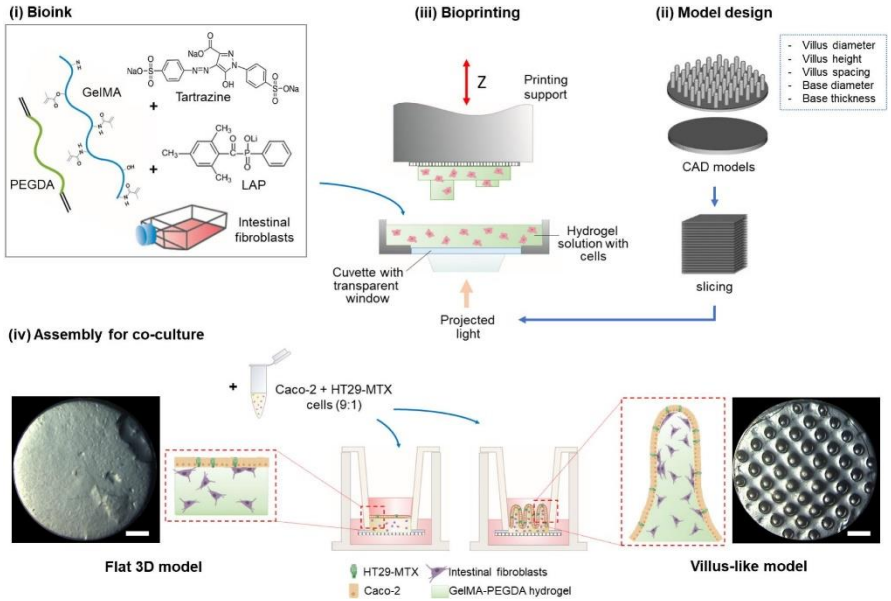
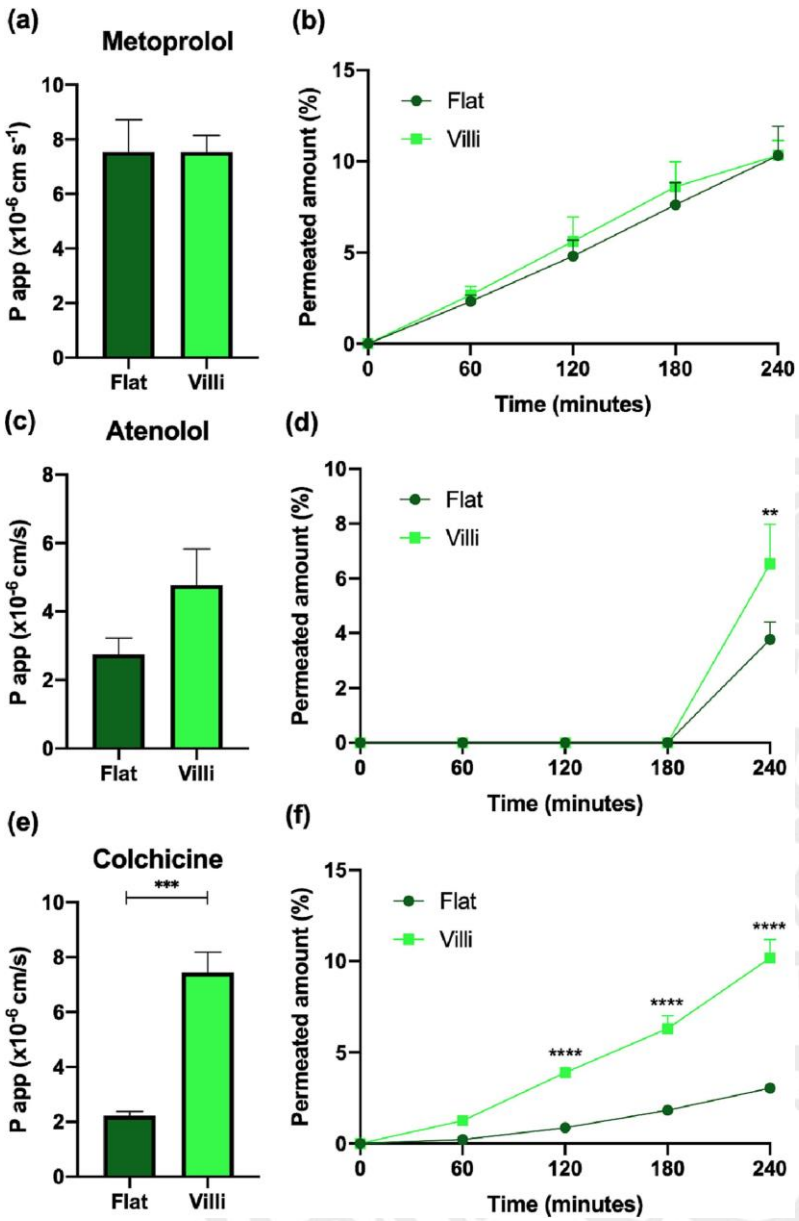
## The shape of our gut: Dissecting its impact on drug absorption in a 3D bioprinted intestinal model

**Macedo MH, et al . Biomater Adv. 2023 20;153:213564.**



# Conclusion

- Regional human intestinal P*eff* identified as one important factor for future determinations.
- It seems as open system provides higher P*eff* than double balloon approach – novel capsule systems (external controlled) and long GI-tube methodologies are developed and tested.
- *In vivo* colon P*eff* of special urgency – but very difficult.
- *In vitro* intestinal P*app*-values in the Ussing and 2D cell monolayer models need scaling and adjustment prior use in PBBM.
- The permeability model is important for the assessment of effect of excipients.
- Future intestinal organoids and 3D bioengineered intestinal models exhibit morphological and physiological features that resemble the native intestinal mucosa.
- New and interesting challenges (MM>700 and logD>5) for novel drug candidate - beyond Lipinski's rule of 5! Require model development!
- It is expected there will be exciting and prosperous time for novel drug delivery systems
- Encequidar (ECD) and Elacridar (ELD) may be very useful as tool for assessment of intestinal efflux (P-gp and BCRP).
- The future role of molecular dynamic simulation and of artificial neural network models to predict the effective permeability of oral drugs before synthesis and even allow for optimization of relevant physicochemical properties of new molecules of interest.