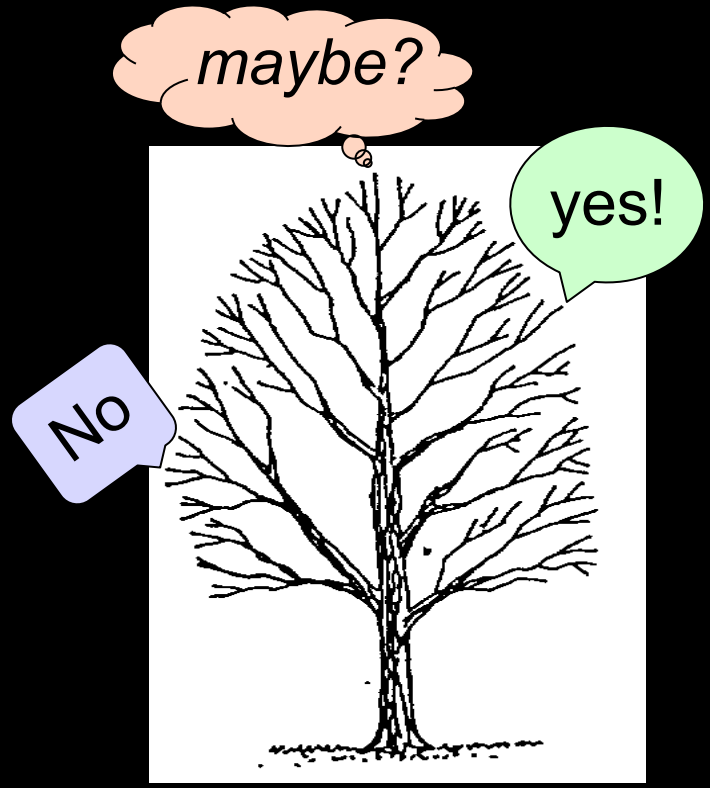
Disposition and Elimination of Organic Anions and Cations: Transporters and Decision Trees



Douglas H. Sweet, PhD

Associate Professor
Department of Pharmaceutics
Virginia Commonwealth University

North Jersey ACS Drug Metabolism Discussion Group
2010 Spring Symposium
April 22, 2010

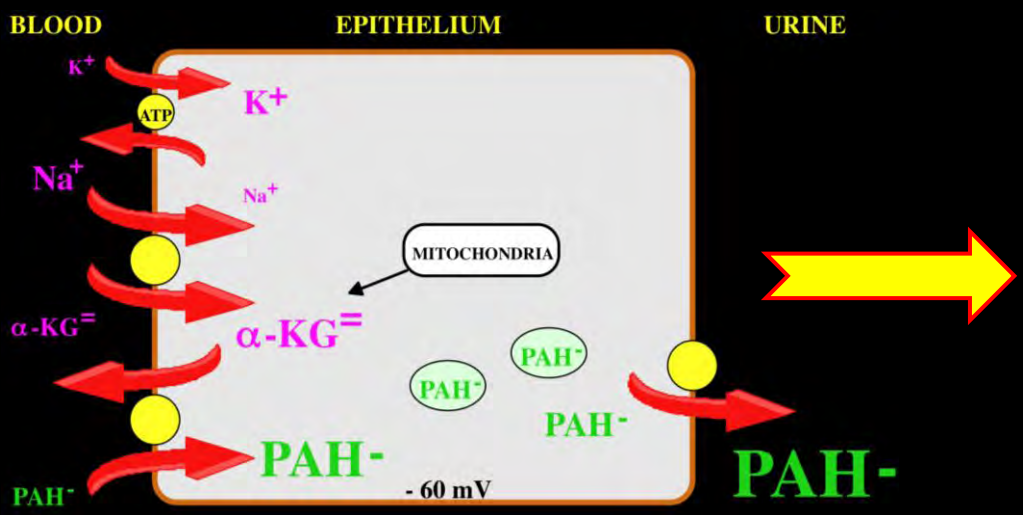
Overview

- How did we get from observing physiological phenomena to studying cloned genes?

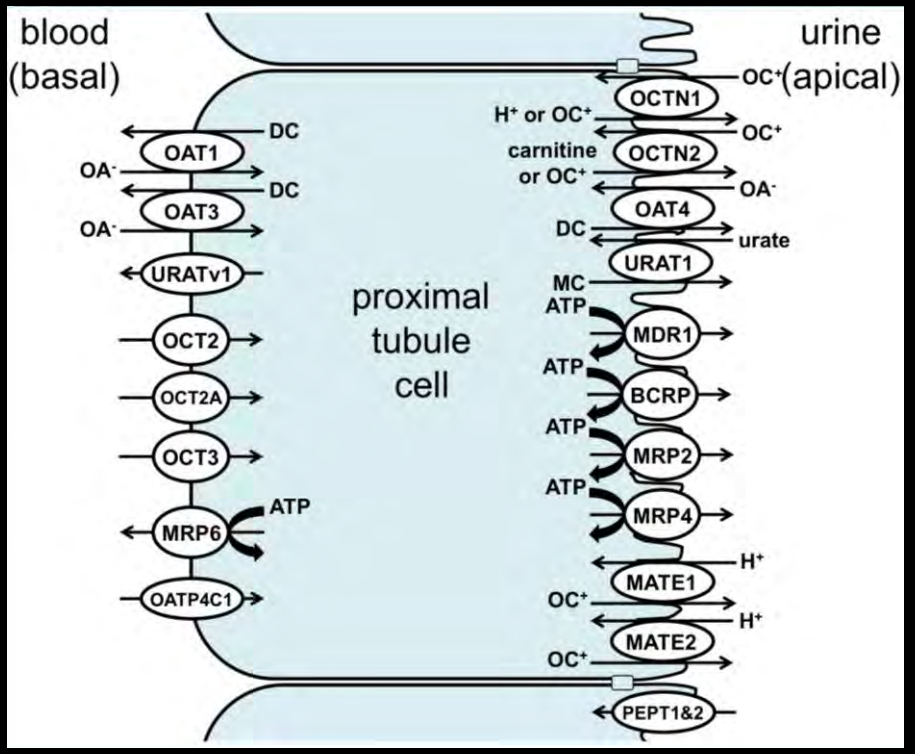
- What are the advantages of having cloned transporters?
  - mechanistic characterization in heterologous expression systems
  - membrane targeting/polarization
  - knockout mouse models

- Do organic anion and cation transporters (OATs/OCTs) impact ADME and human disease states?

Renal Transport Then and Now

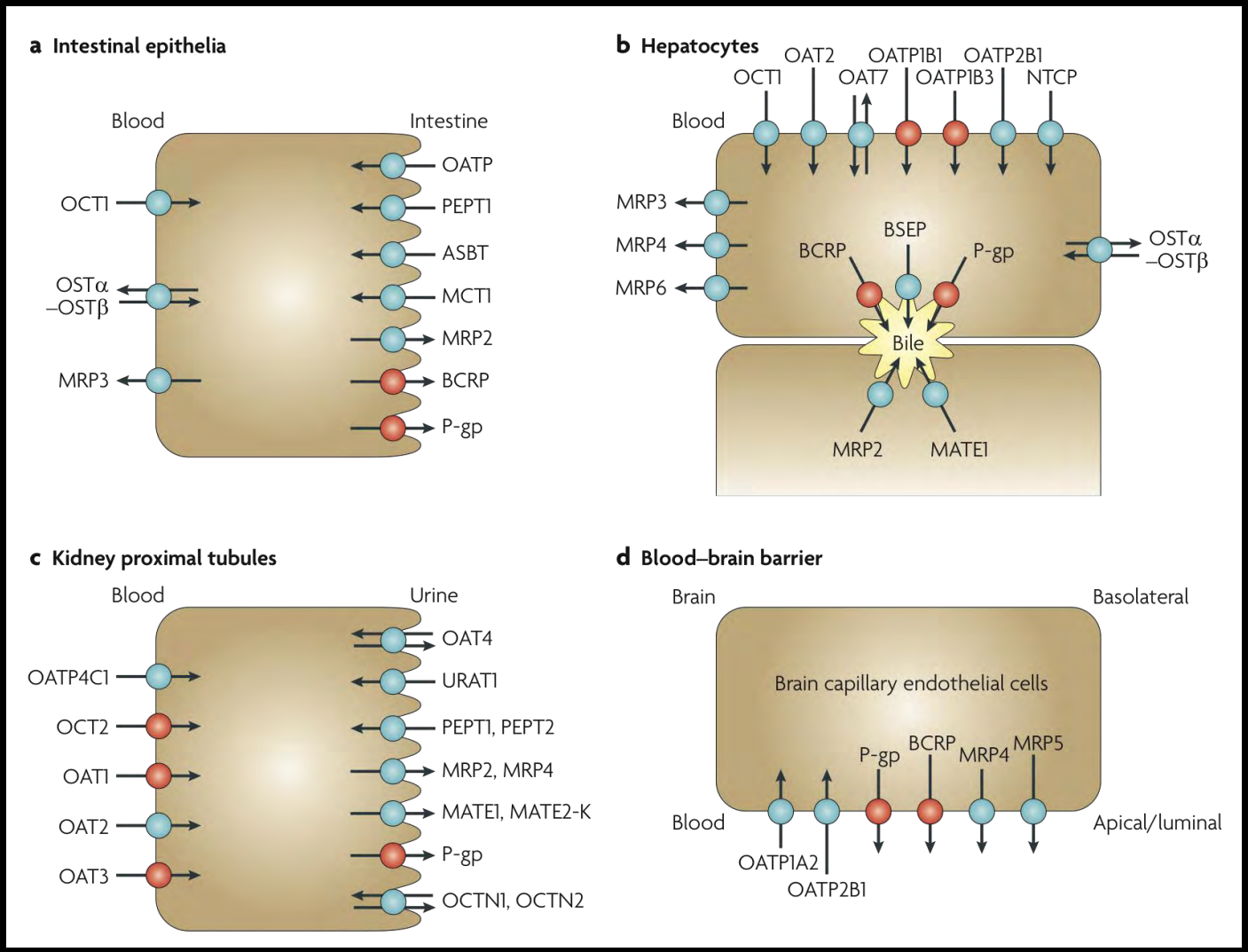


circa 1996



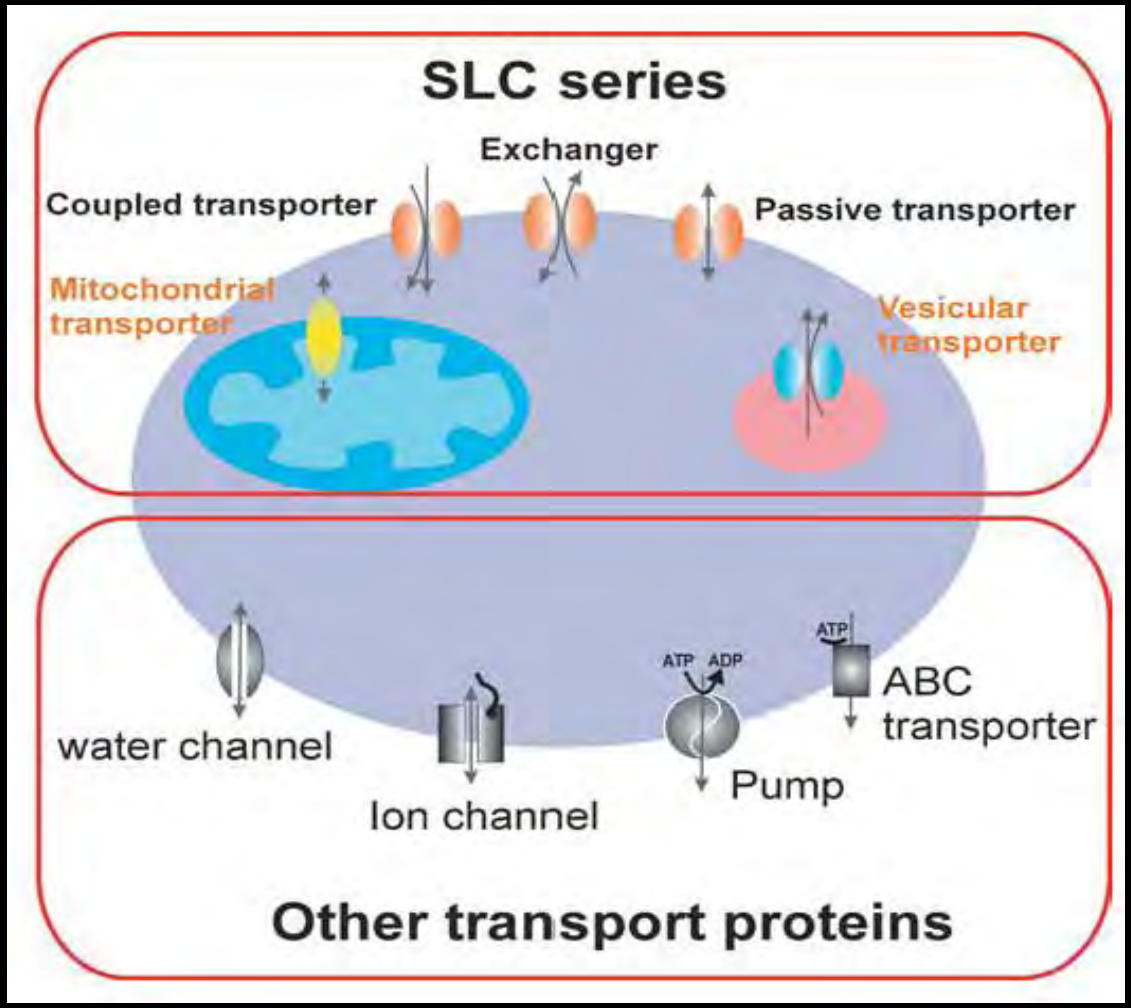
2010

“Renal” Transporters = Barrier Membrane Transporters



ITC Whitepaper, *Nat Rev Drug Discov* 9, 2010

Transport Proteins



Hediger et al., Pflug Arch 447, 2004

Expression Cloning Strategy

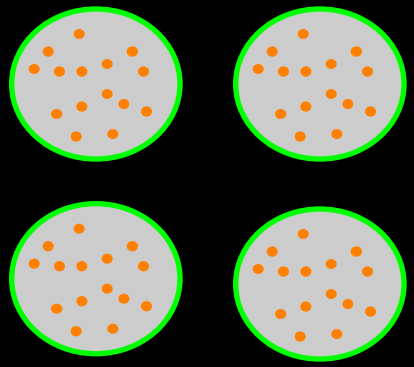


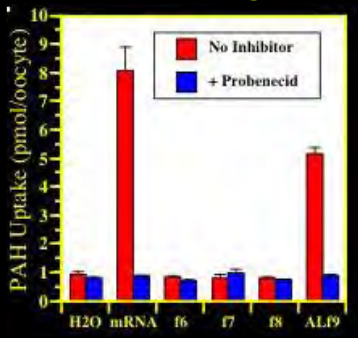
Plate Bacteria Containing cDNA Library

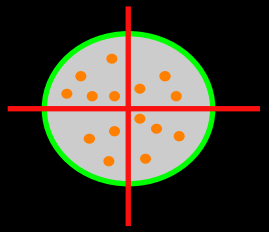
Purify Plasmid DNA

in vitro Transcription [cRNA]

Inject *Xenopus* oocytes

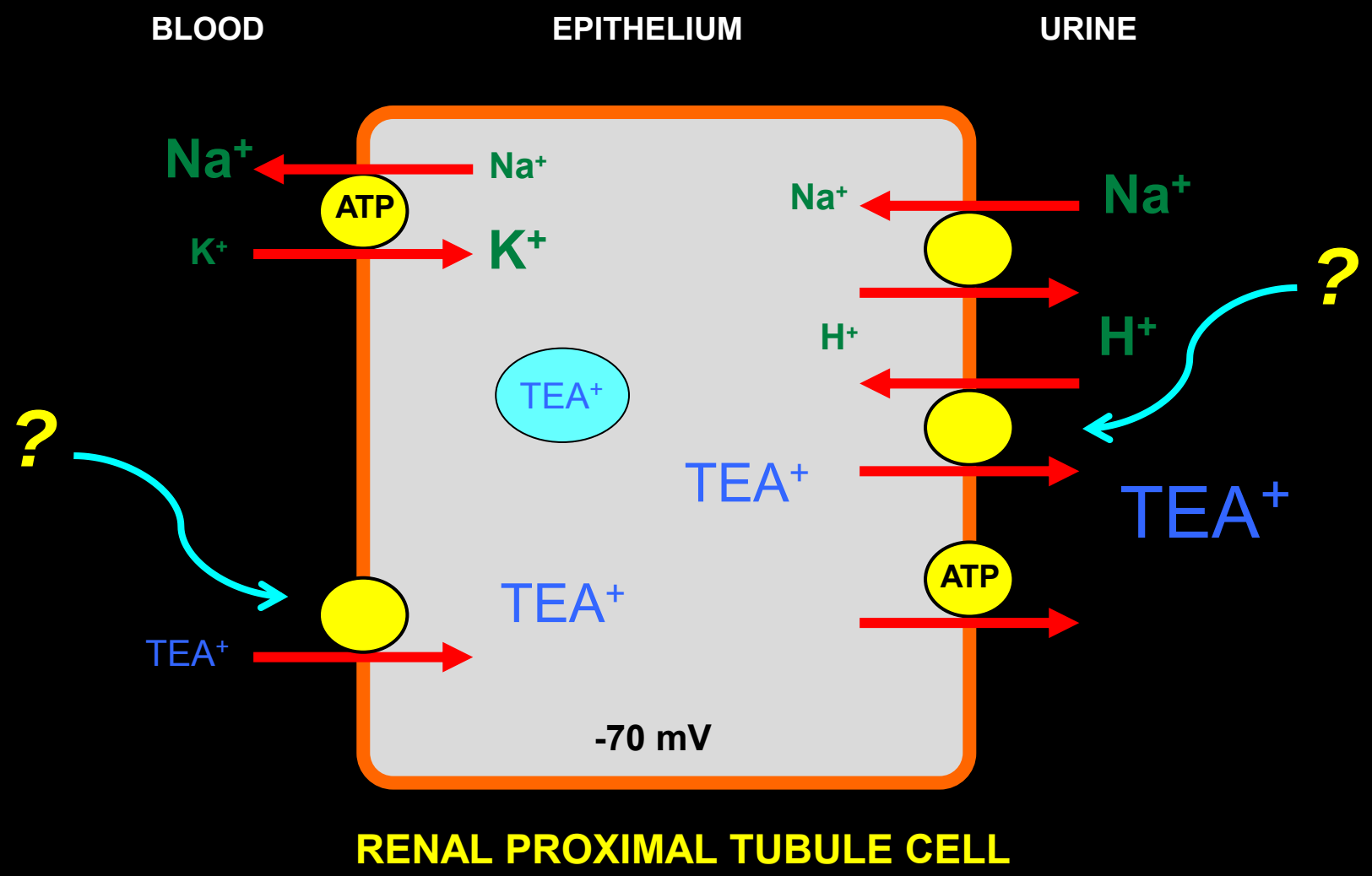
Expression Assay



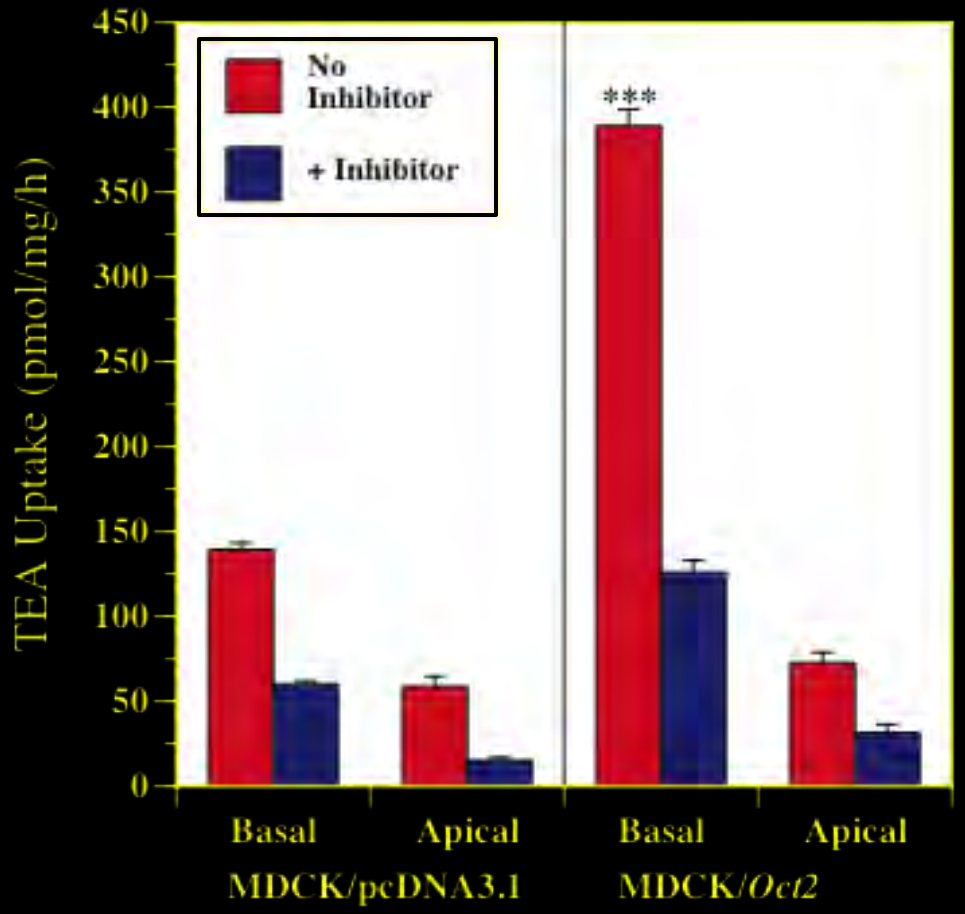


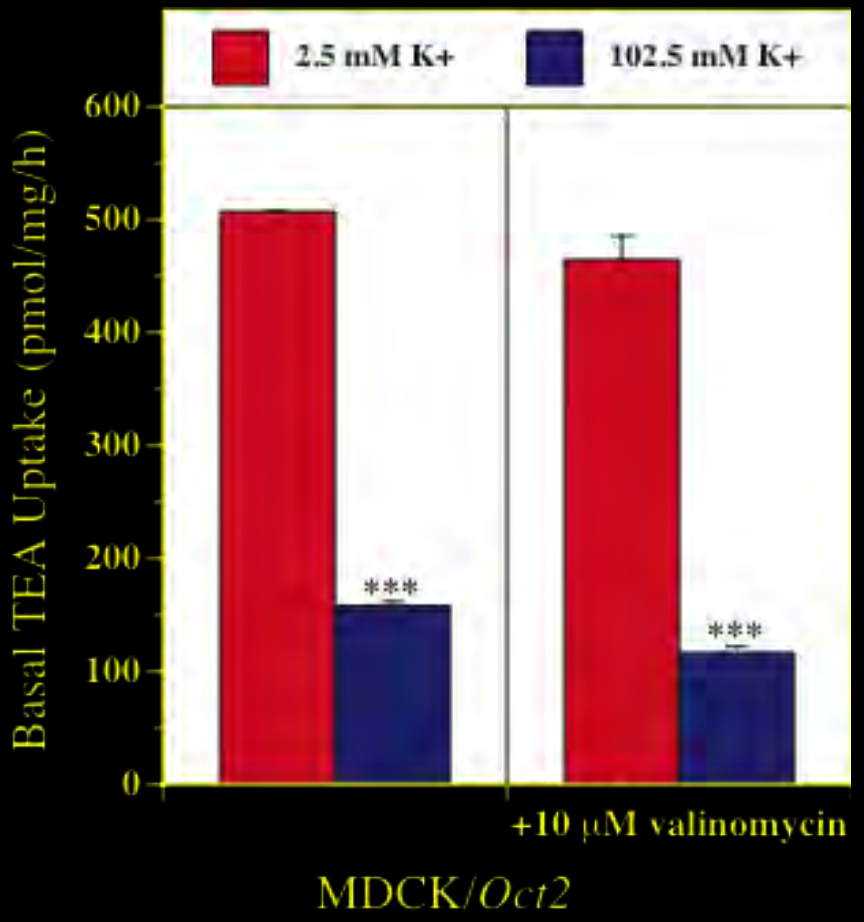
Subsection Positive Filter

Where Does the Cloned Transporter go?



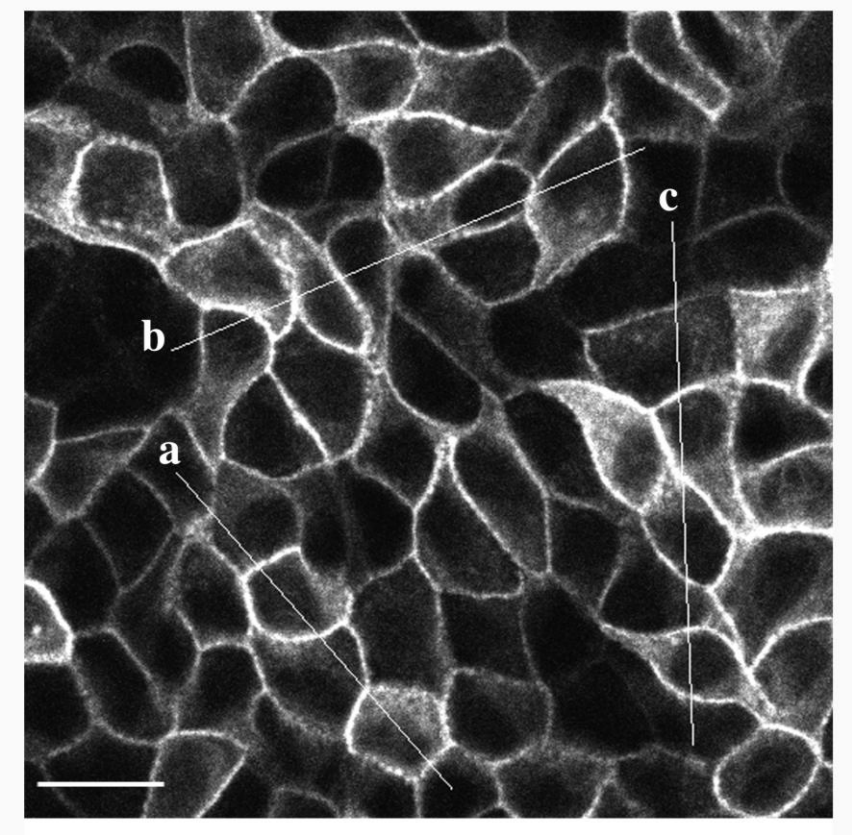
MDCK-Oct2 TEA Transport

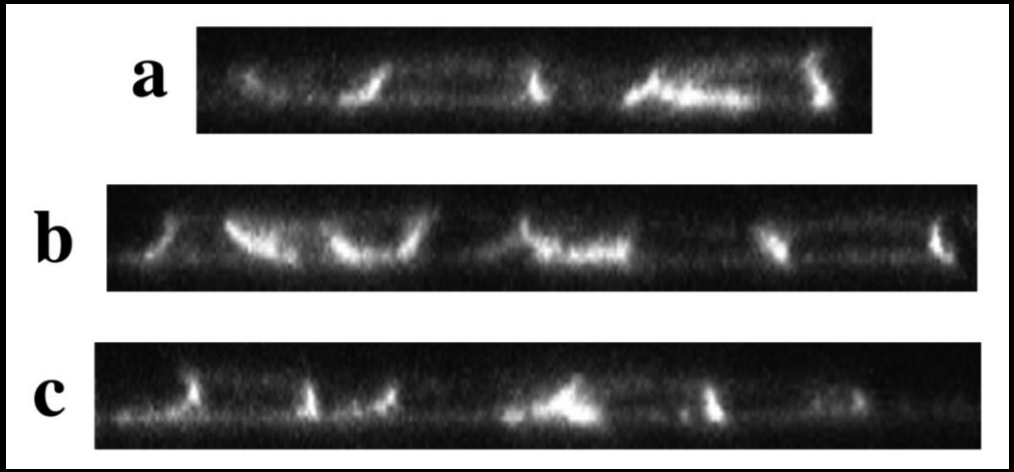




Sweet et al., *Am J Physiol Renal Physiol* 277, 1999

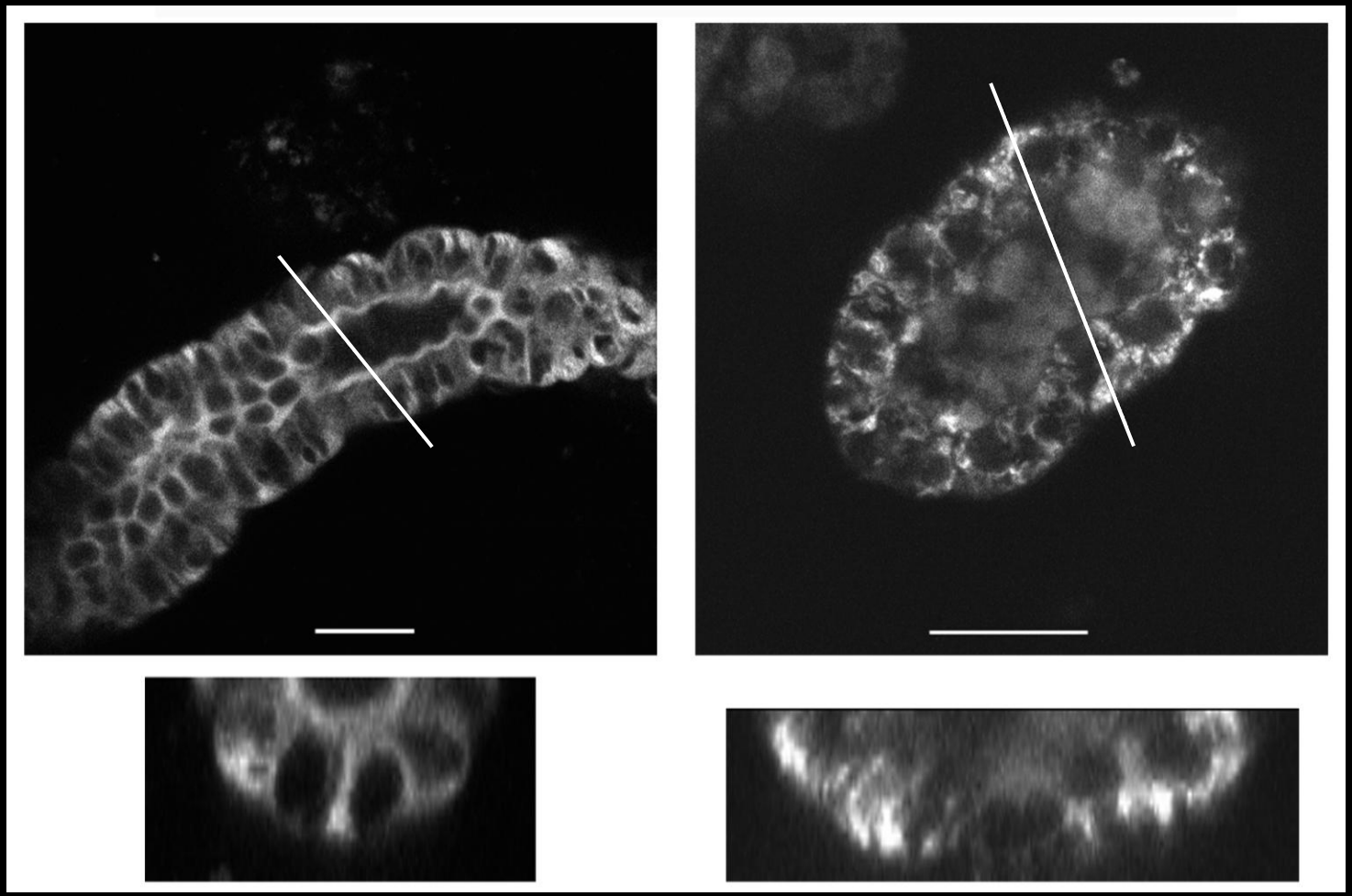
Oct2-GFP Targeting in MDCK Cells





Sweet et al., *Am J Physiol Renal Physiol* 279, 2000

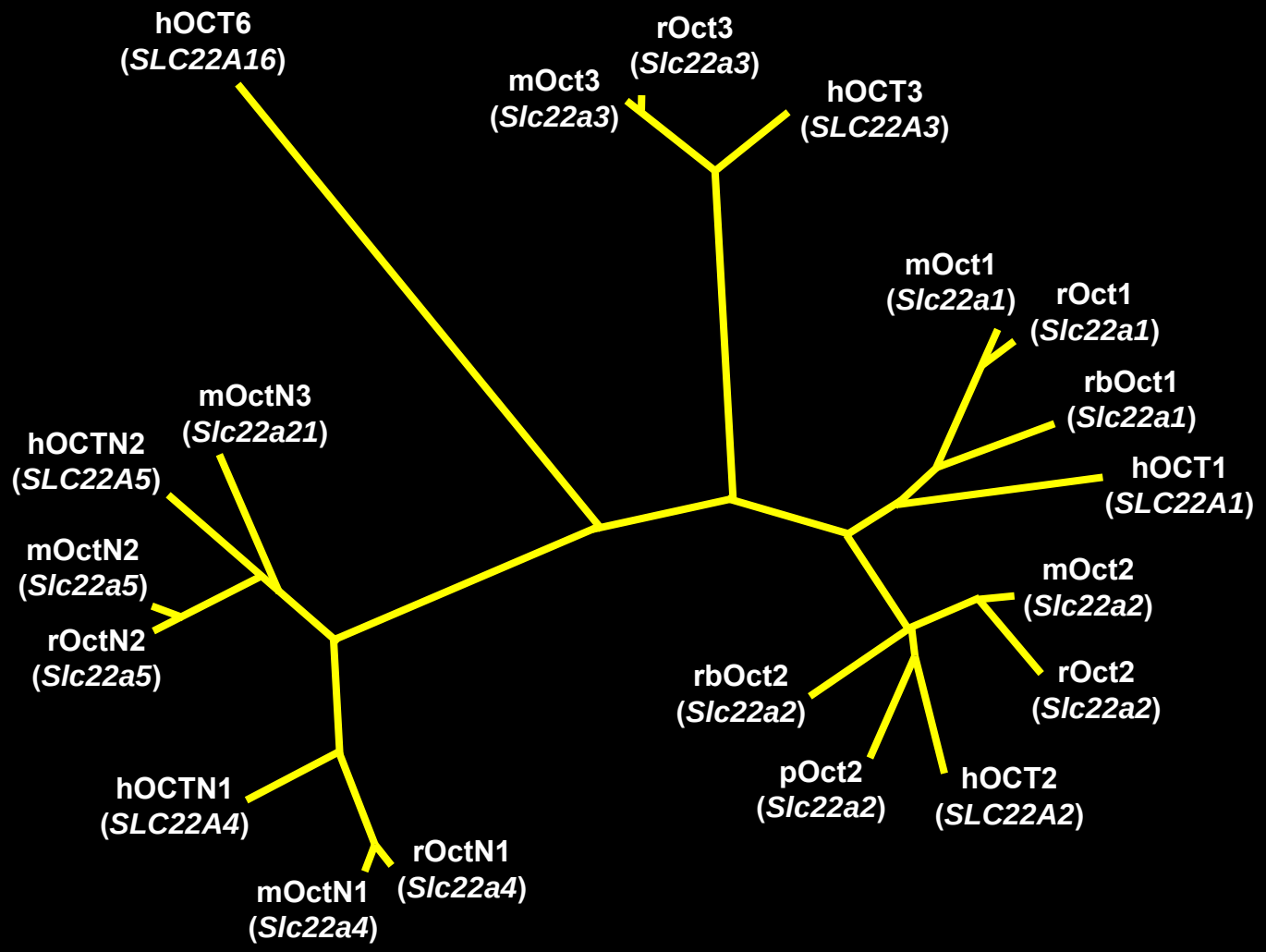
Oct2-GFP Targeting in Killifish Renal Tubules



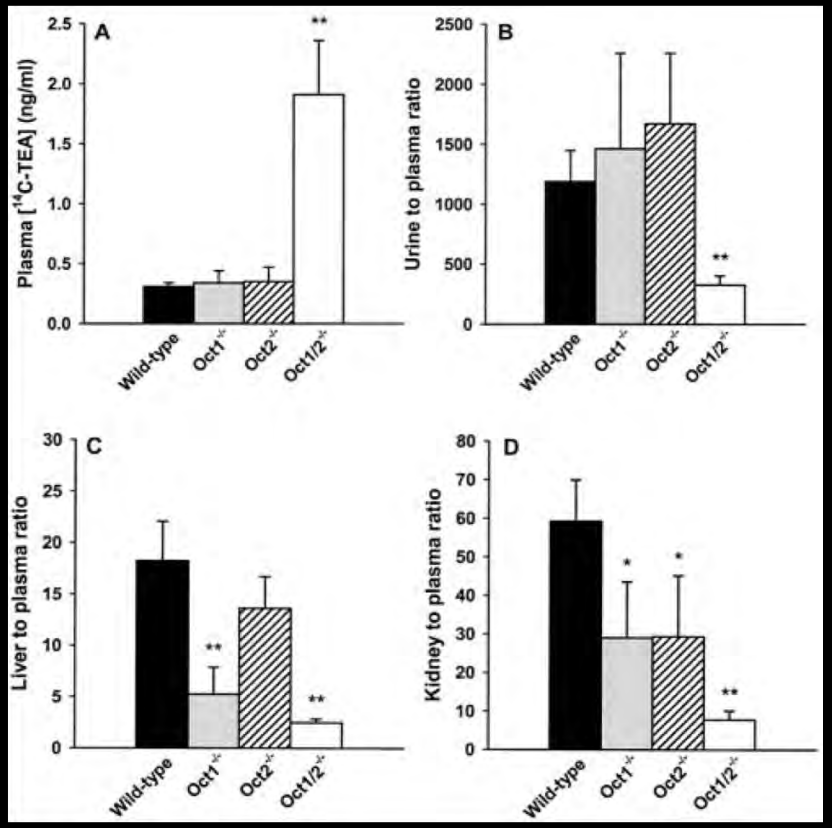
FM 4-64 rOct2-GFP

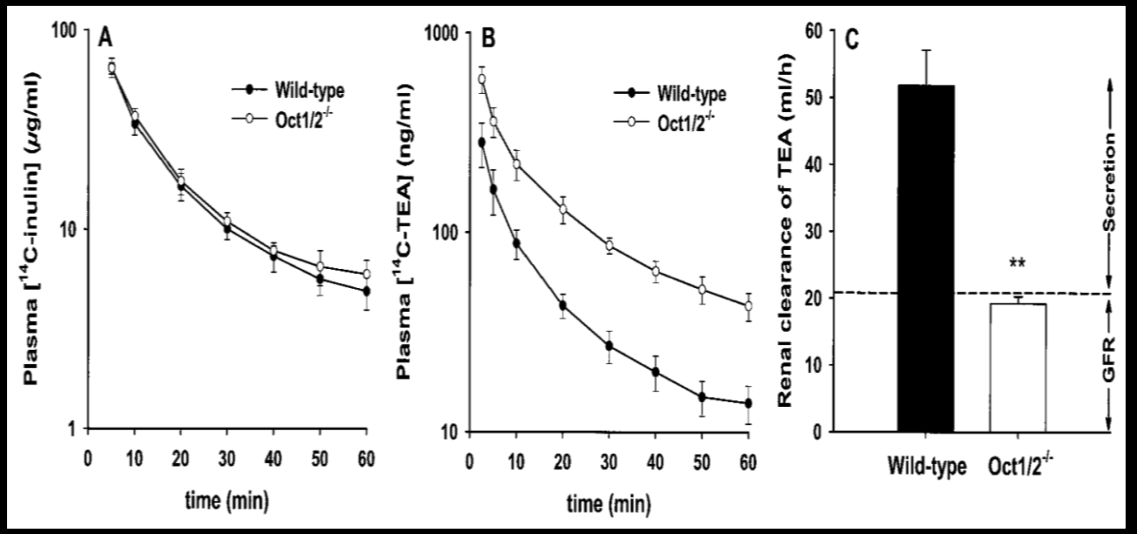
Sweet et al., *Am J Physiol Renal Physiol* 279, 2000

Organic Cation Transporter Family



Oct1,Oct2 and Oct1/2 Knockout Mice

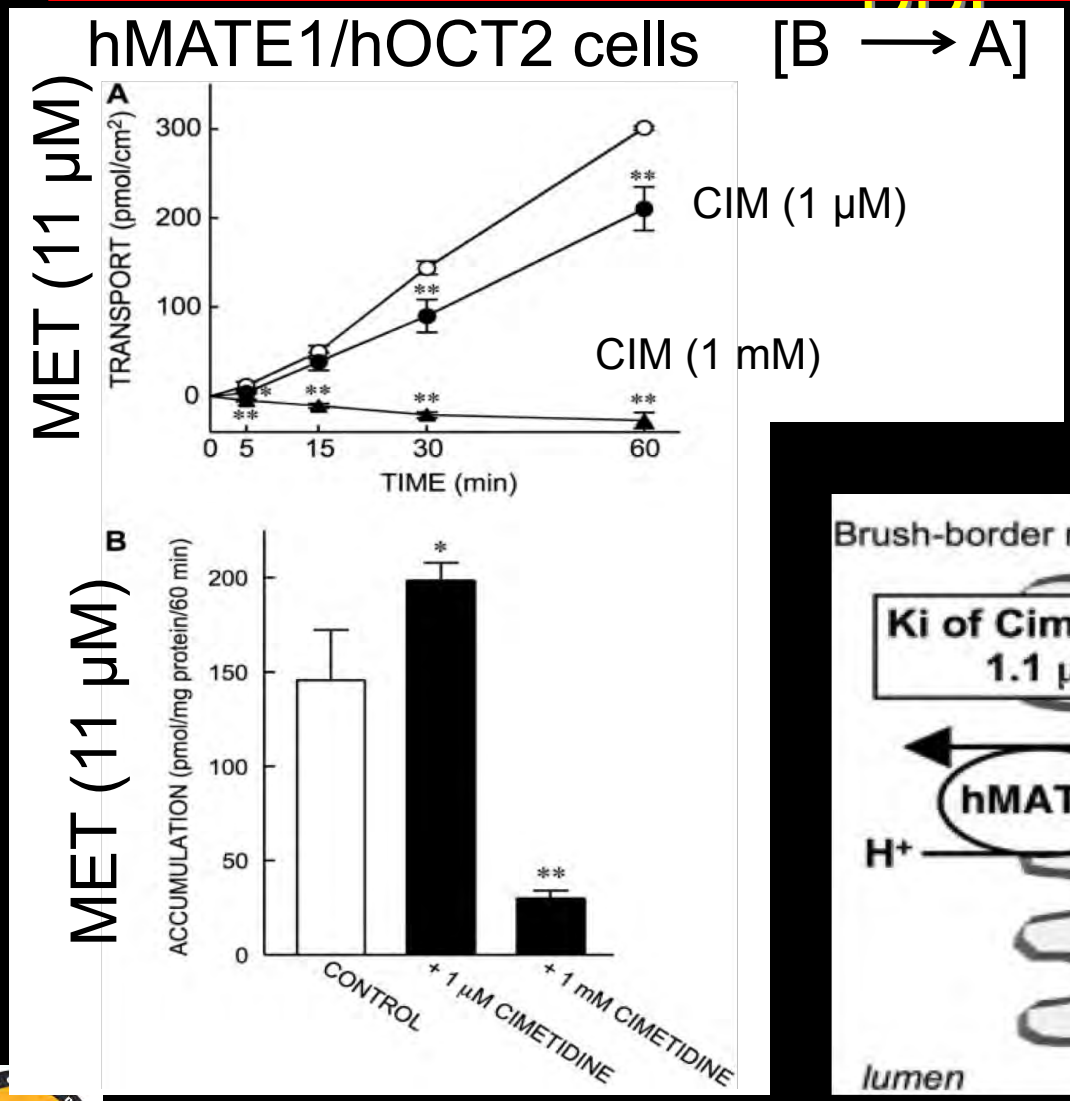




Jonker et al., *Mol Cell Biol* 21, 2001

Jonker et al., *Mol Cell Biol* 23, 2003

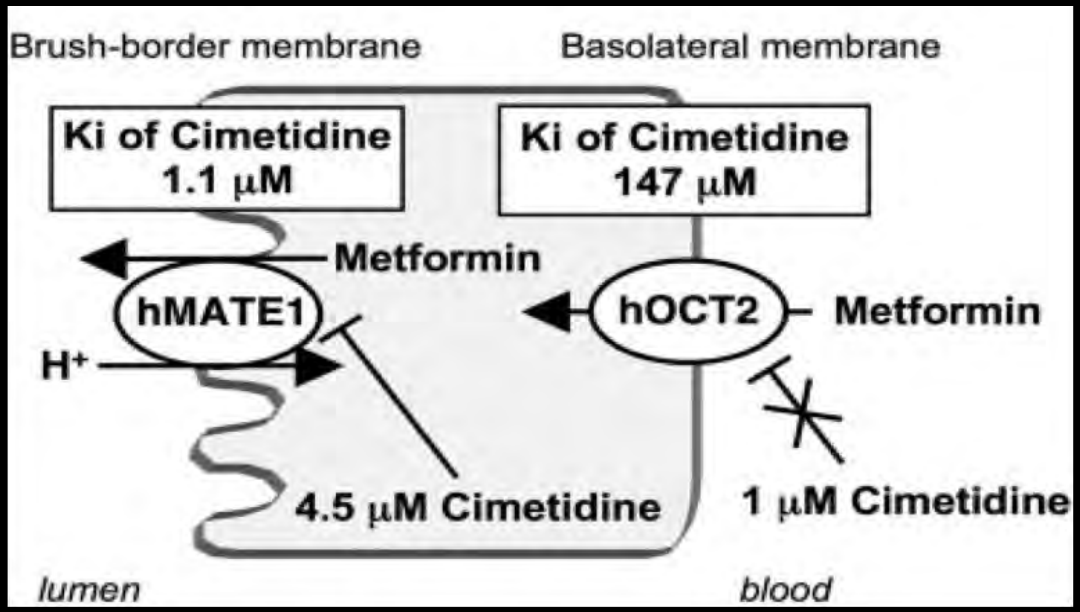
Renal Transport Pathway Mediating Metformin/Cimetidine DDI



K_i for Cimetidine:

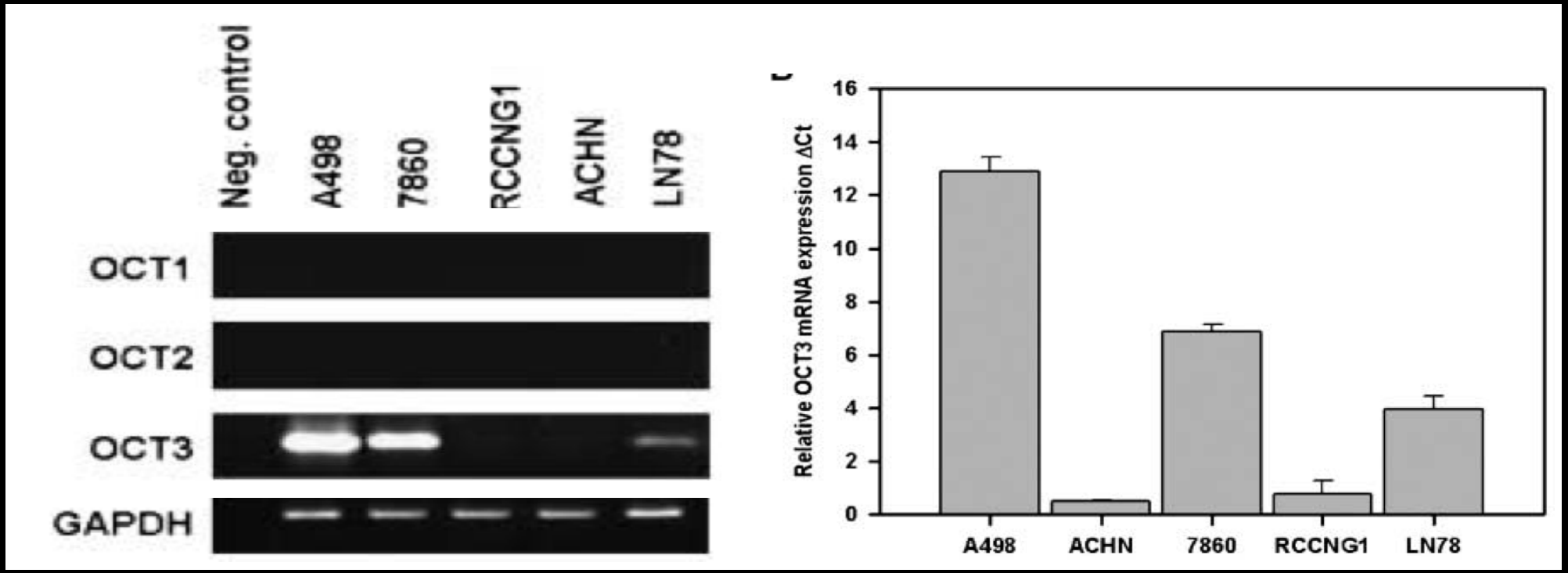
hOCT2 ≅ 150 μM

hMATE1≅ 1 μM



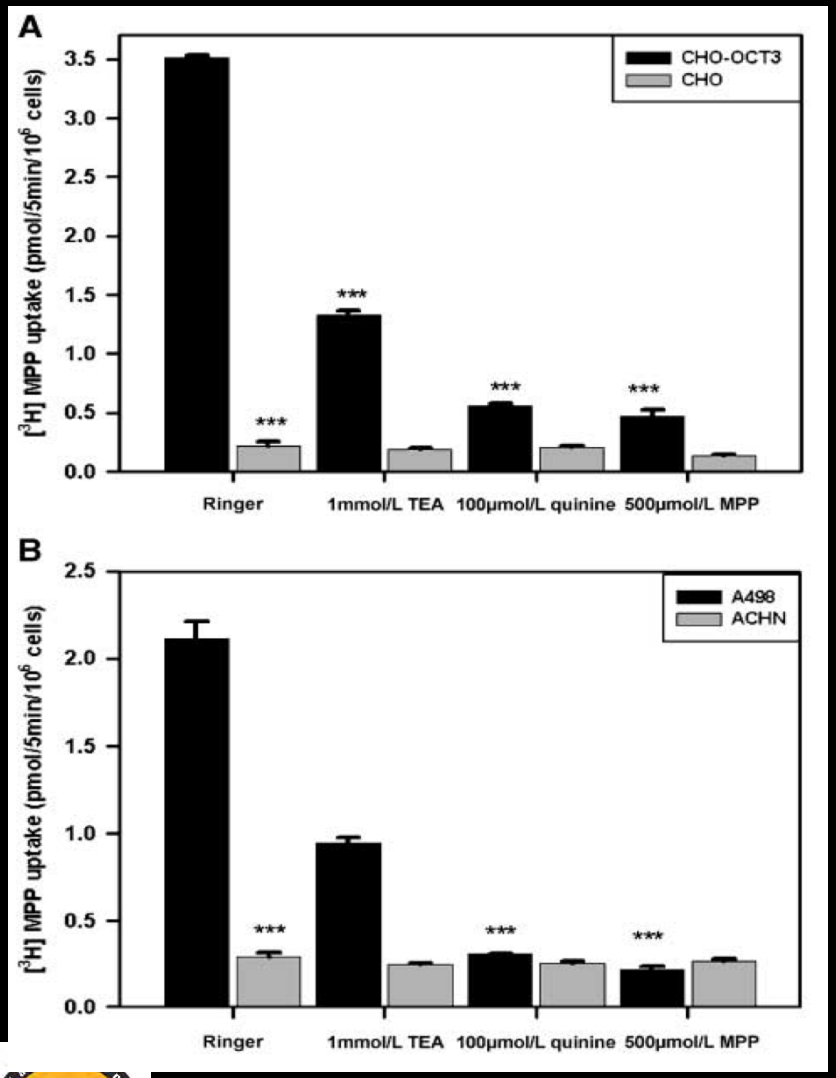
Tsuda, et al. *JPET* 329, 2009

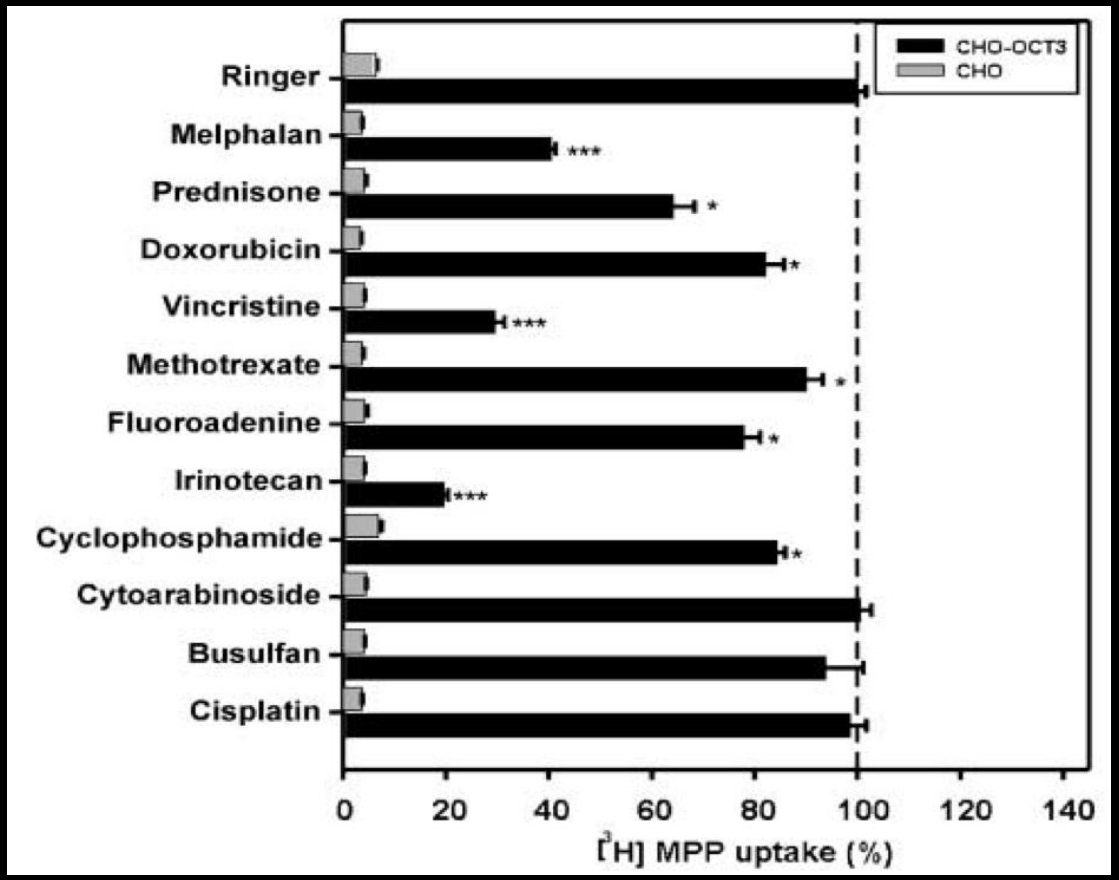
OCT3 Expression in Renal Cell Carcinoma



Shnitsar, et al. *Cancer Res* 69(4), 2009

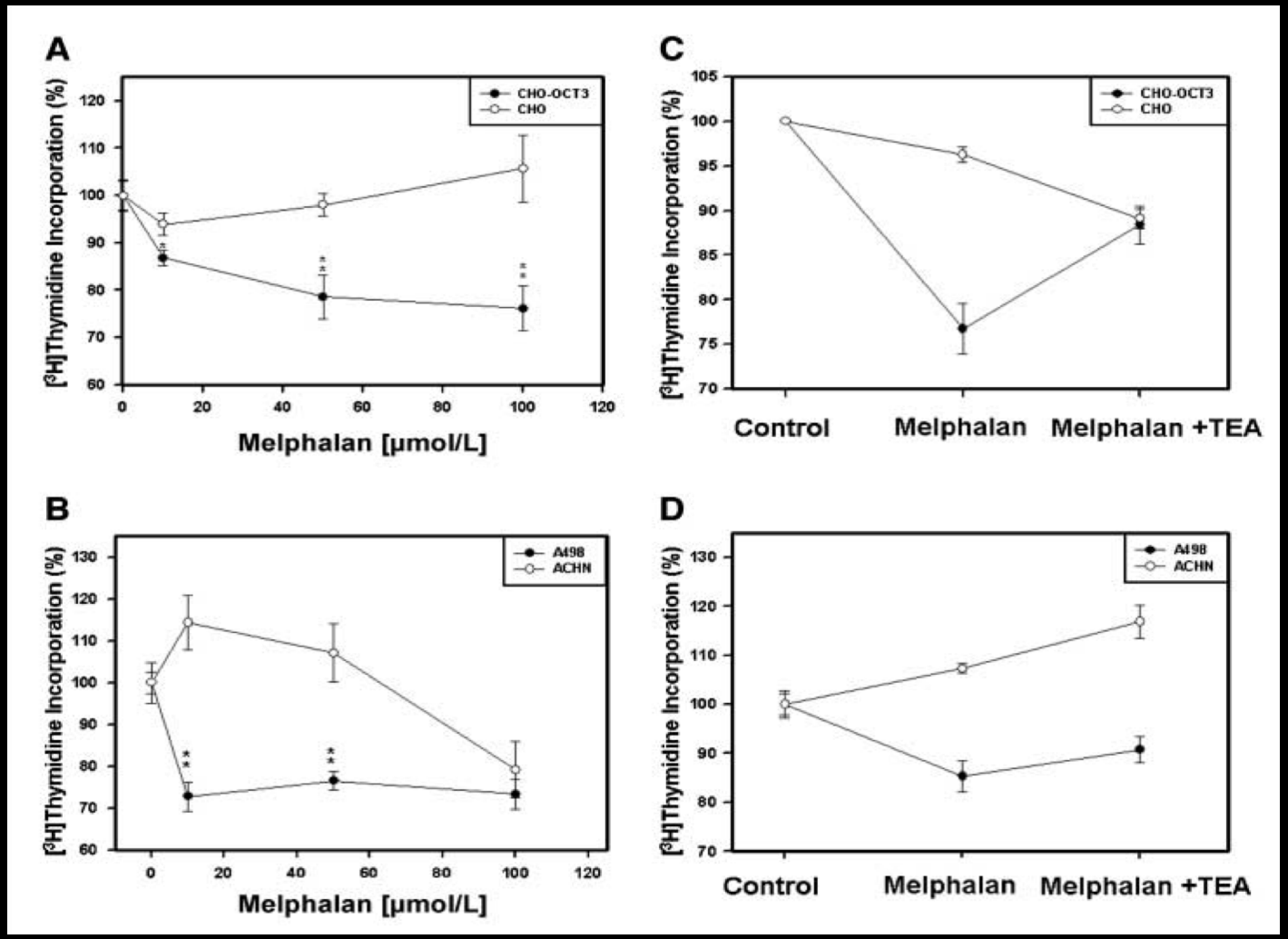
OCT3 Function and Renal Cell Carcinoma





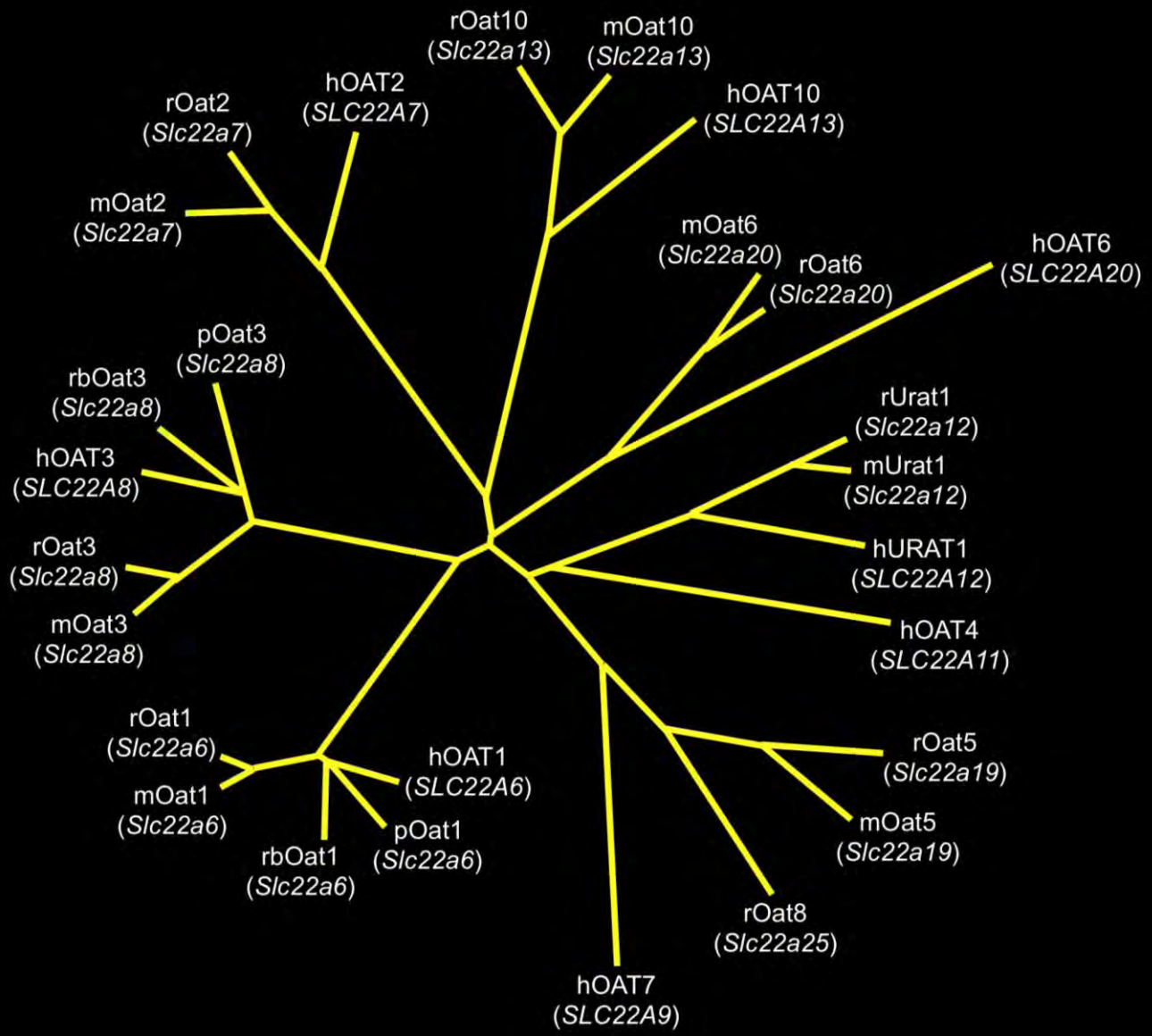
Shnitsar, et al. *Cancer Res* 69(4), 2009

OCT3 Function and Renal Cell Carcinoma

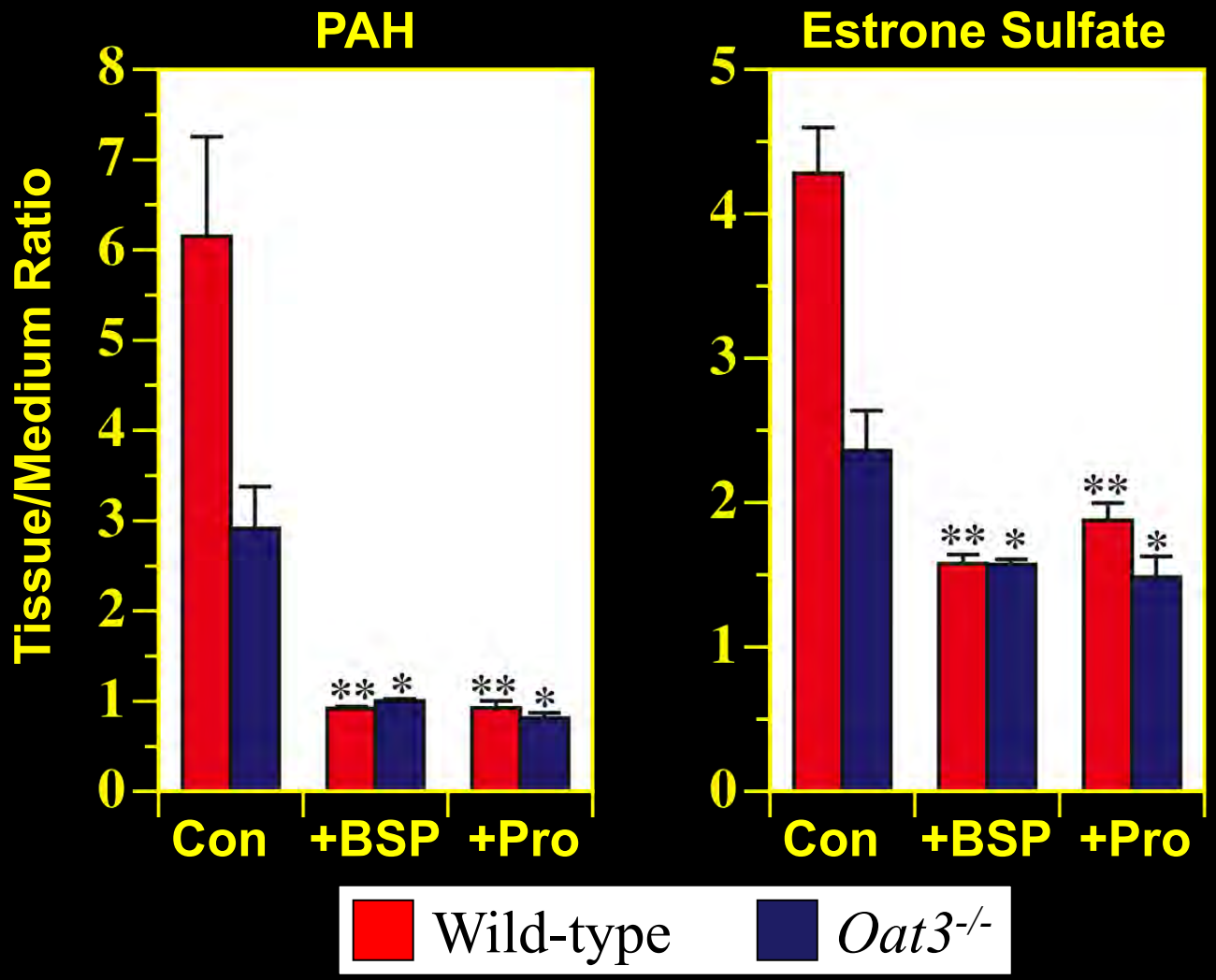


Shnitsar, et al. *Cancer Res* 69(4), 2009

Organic Anion Transporter (SLC22) Family

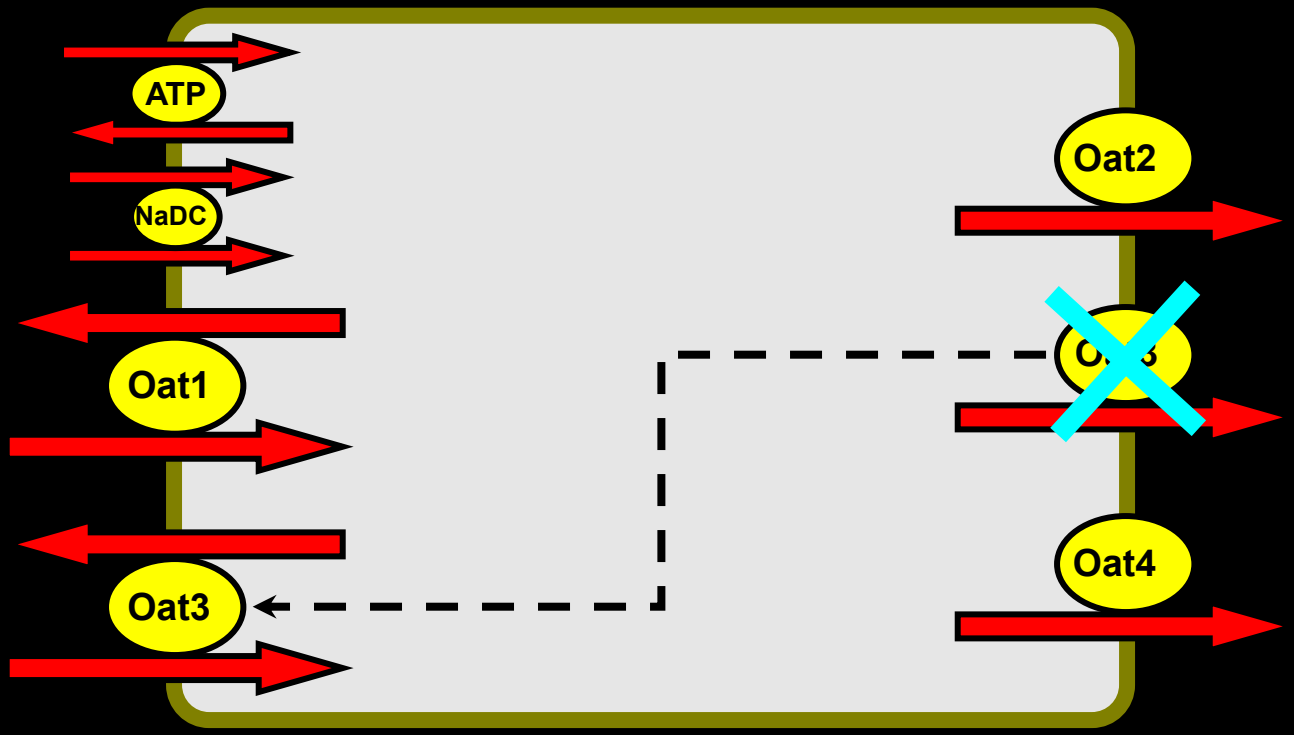


Uptake in Renal Slices From Oat3$^{(-/-)}$ Mice



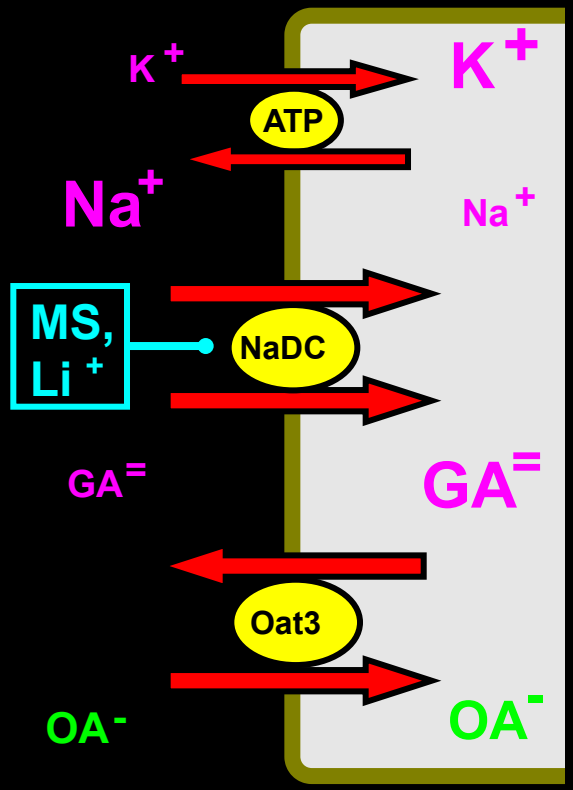
Sweet, et al. (2002) *J Biol Chem* 277(30): 26934-26943

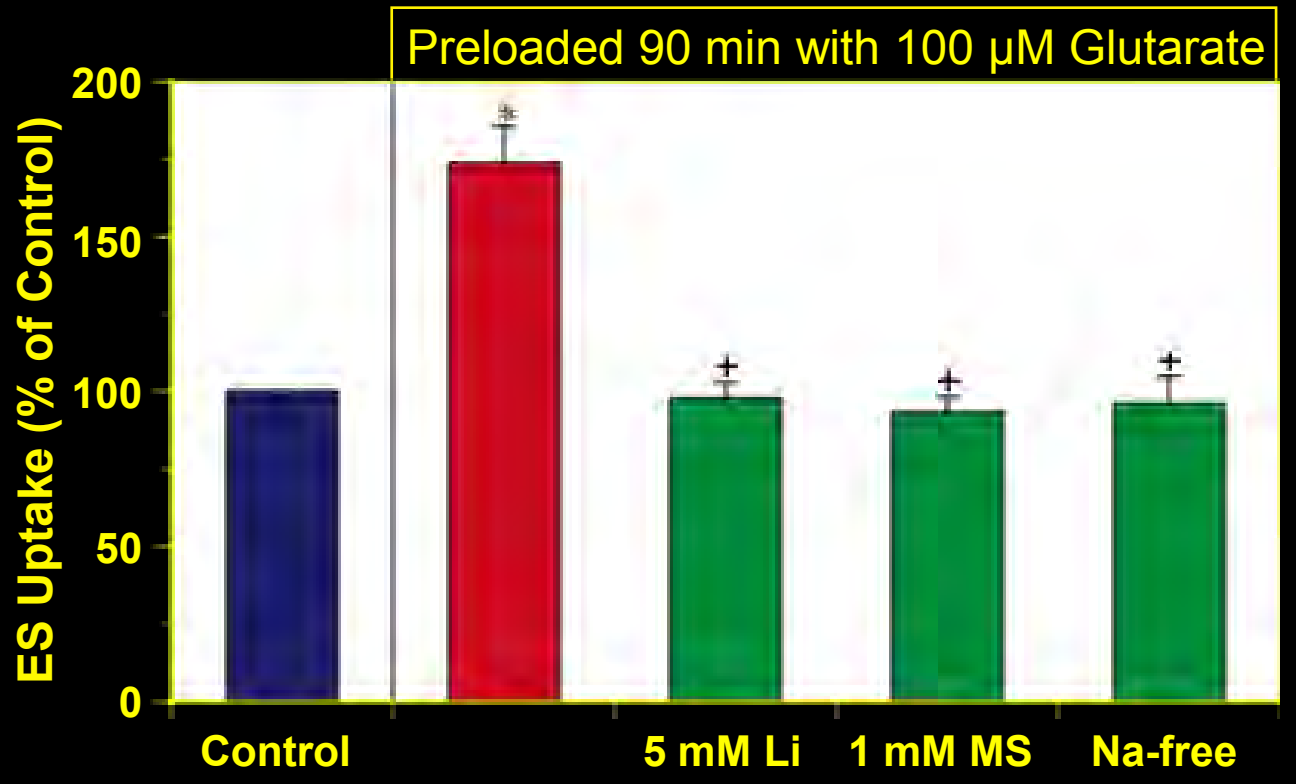
Proposed Change to Transport Model



Oat3 localized to basal membrane; mechanism should be reevaluated

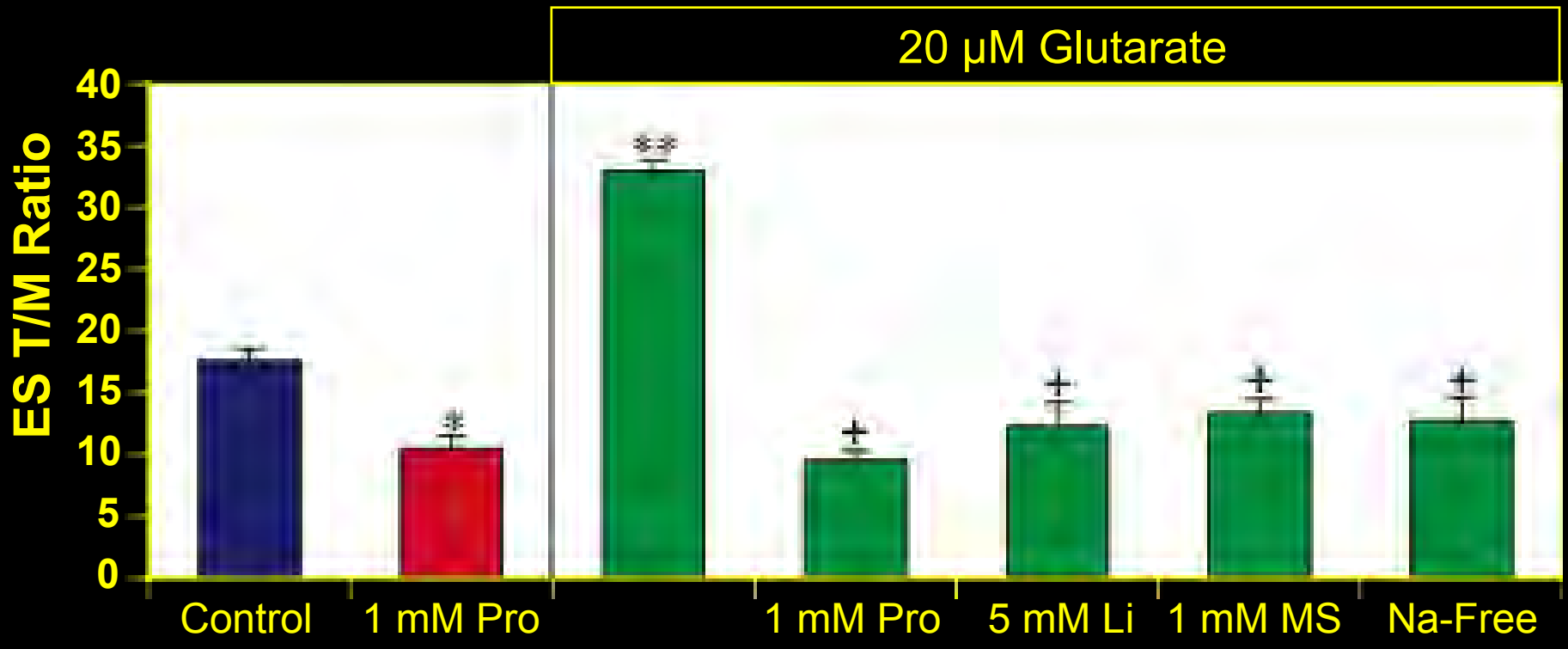
Uptake in Oocytes Co-injected with Oat3 and NaDC cRNAs





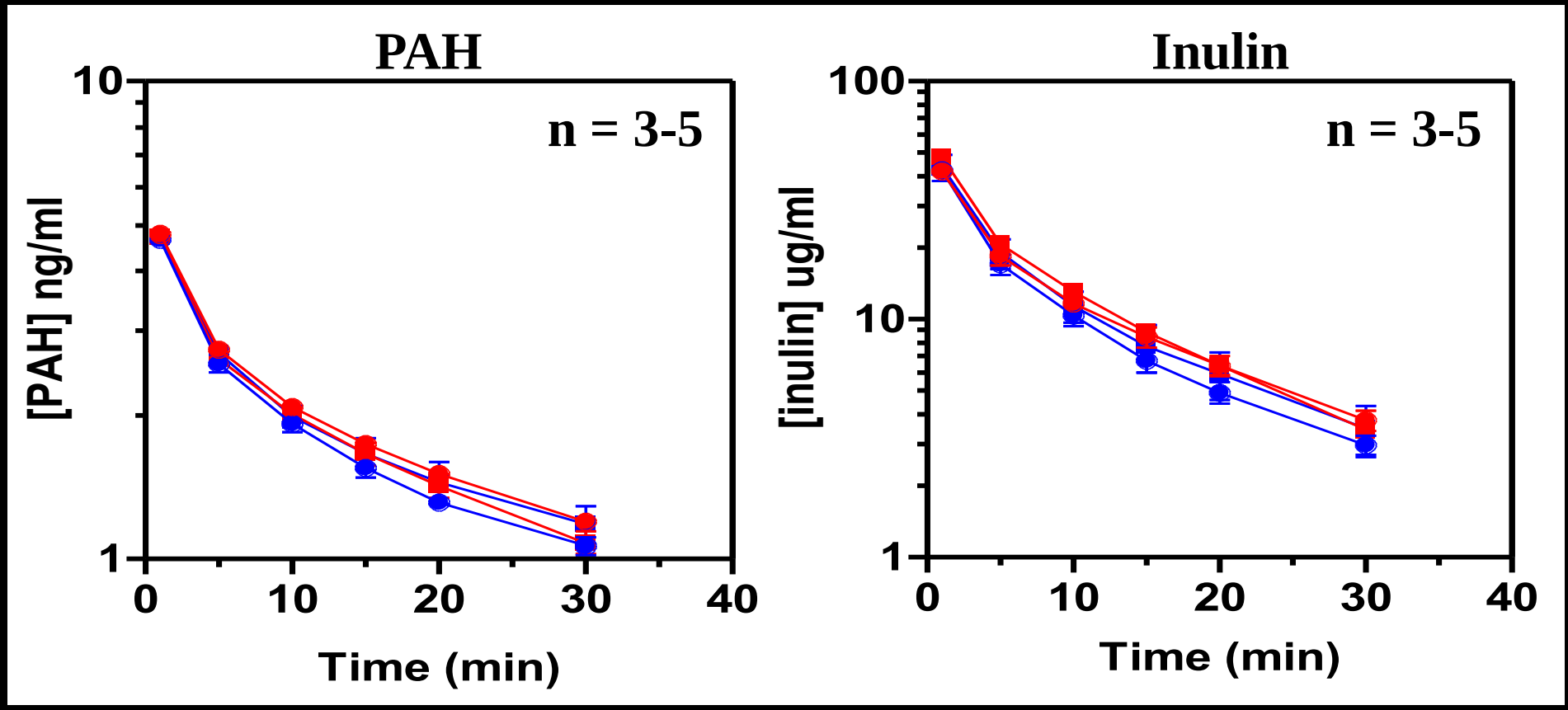
Like Oat1, Oat3-mediated uptake is energetically coupled to the Na^+ gradient through the Na^+/dicarboxylate cotransporter

ES Uptake in Renal Cortical Slices



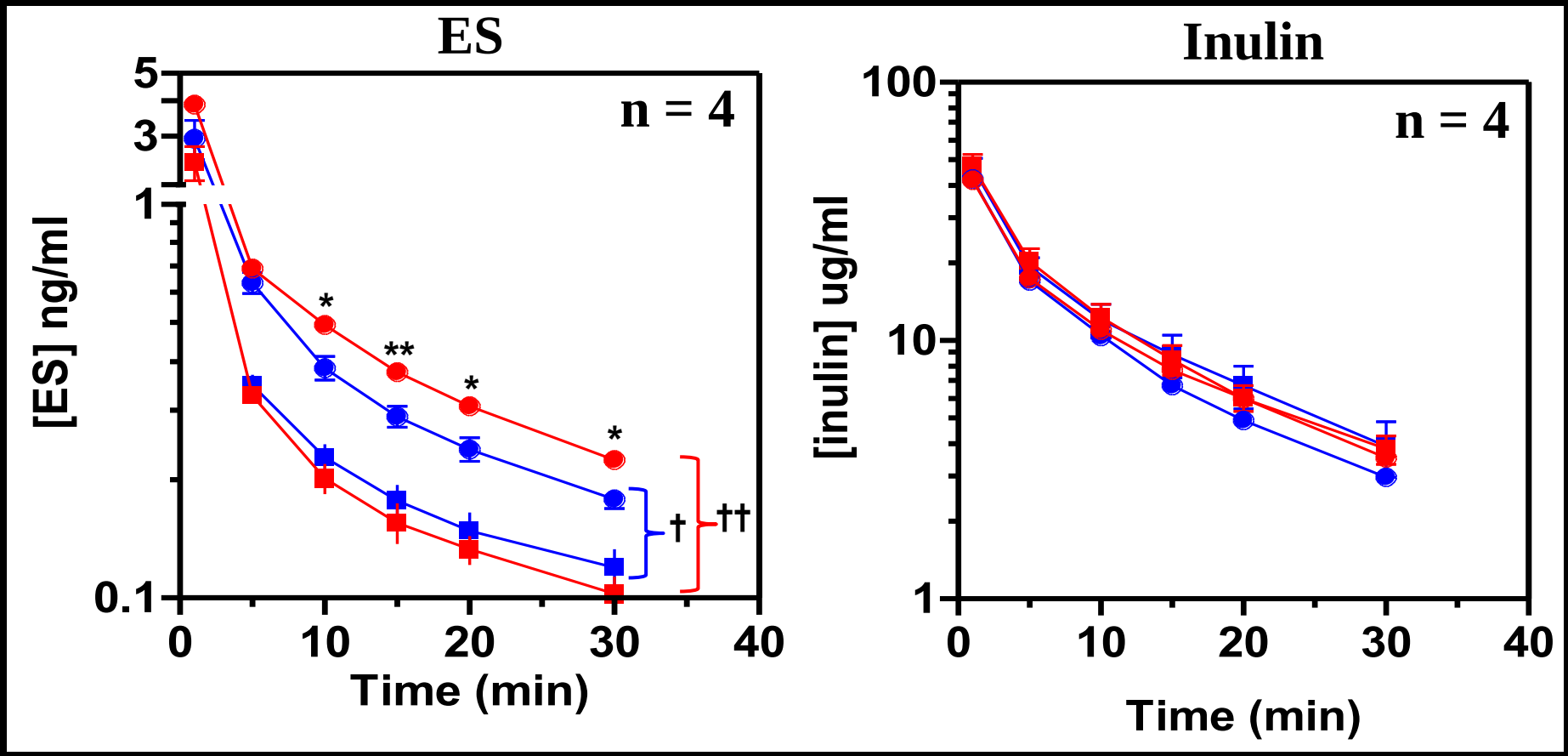
Oat3-mediated renal basolateral ES uptake is energetically coupled to the Na^+ gradient through the Na^+/dicarboxylate cotransporter

PAH Elimination is Not Altered in *Oat3*$^{(-/-)}$ Mice



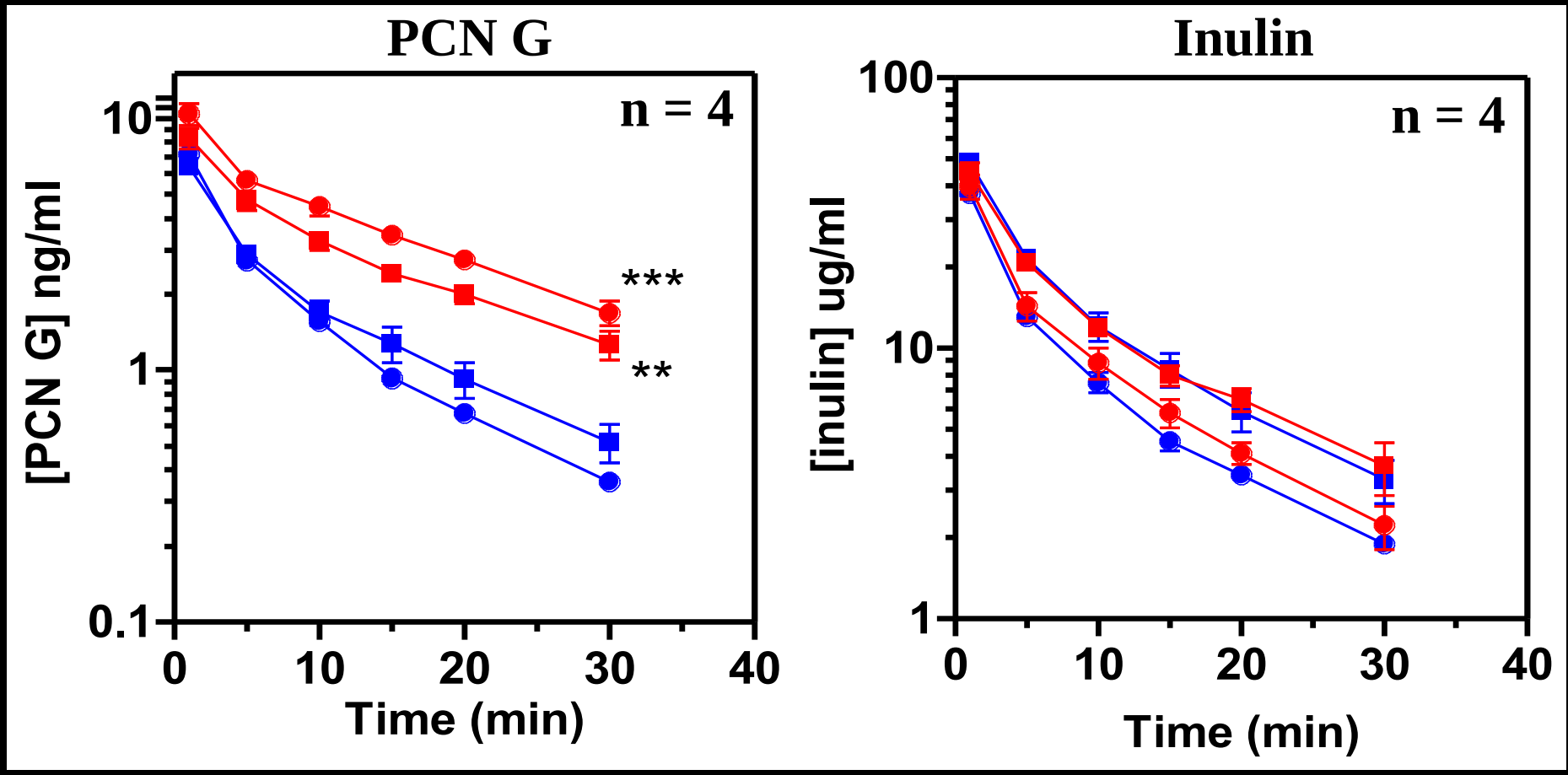
VanWert, et al. (2007) *Am J Physiol Renal Physiol* 293: F1332-F1341

Impaired ES Elimination in Female *Oat3*$^{(-/-)}$ Mice



VanWert, et al. (2007) *Am J Physiol Renal Physiol* 293: F1332-F1341

Markedly Impaired PCN G Elimination in *Oat3*$^{(-/-)}$ Mice

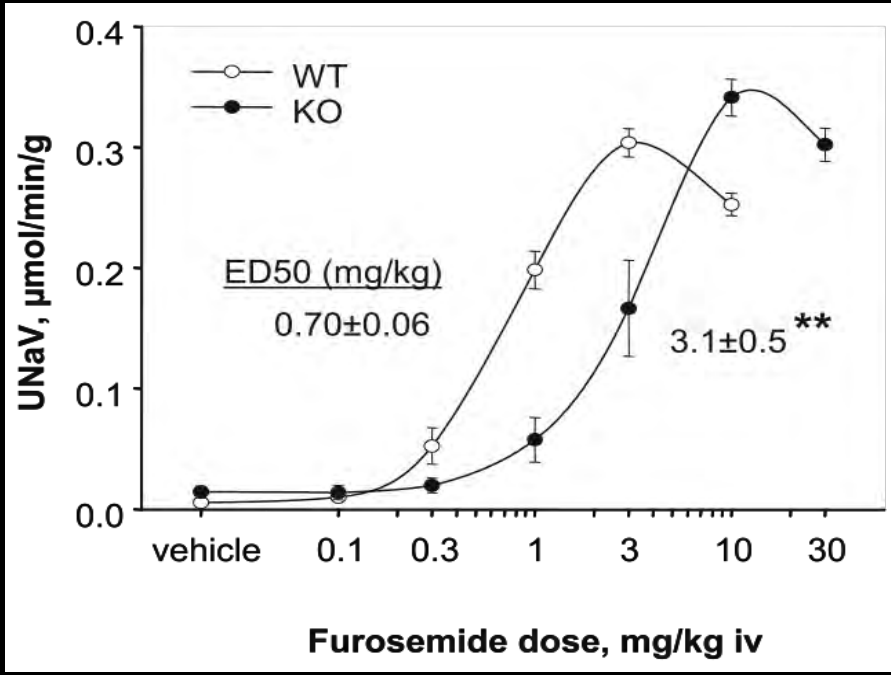


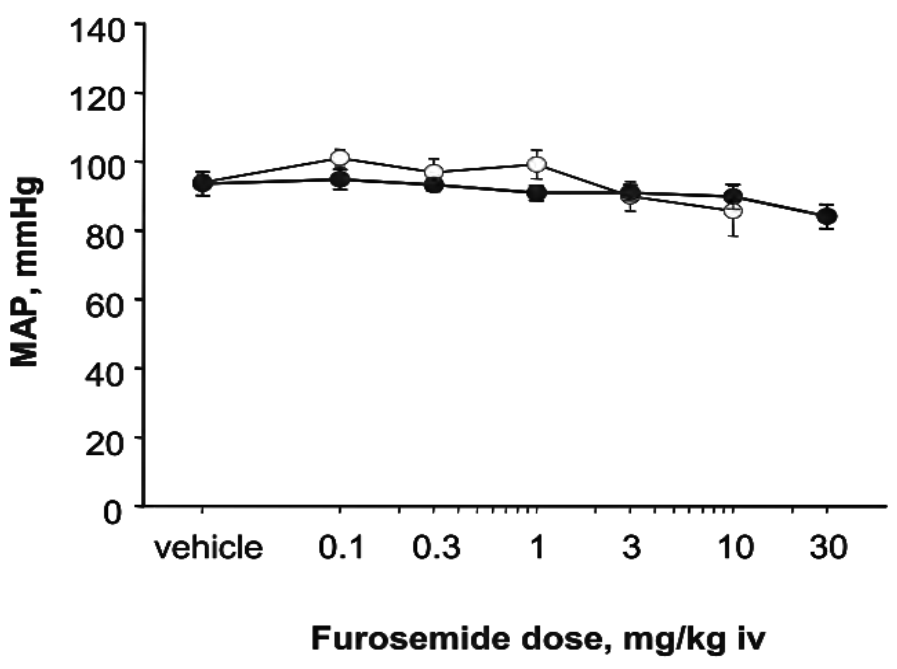
VanWert, et al. (2007) *Am J Physiol Renal Physiol* 293: F1332-F1341

Pharmacokinetic Changes in *Oat3*$^{(-/-)}$ Mice

Sex	Compound	Genotype	Kinetic Parameter: V1 (ml/kg)	Vss (ml/kg)	AUC PAH, ES, & PCNG (min*ng/ml) Inulin (min*ug/ml)	Total Clearance (ml/min/kg)
Male	PAH (1.430 µg/kg)	wild-type (n = 3)	249 ± 12	516 ± 33	113 ± 14	13 ± 1.5
		Oat3(-/-) (n = 3)	231 ± 28	477 ± 46	98 ± 7.0	15 ± 1.1
	ES (0.526 µg/kg)	wild-type (n = 4)	147 ± 22	786 ± 78	14 ± 1.8	39 ± 4.8
		Oat3(-/-) (n = 4)	113 ± 20	682 ± 104	14 ± 1.7	39 ± 4.6
	PCN G (1.870 µg/kg)	wild-type (n = 4)	213 ± 14	406 ± 20	65 ± 8	29 ± 3.6
		Oat3(-/-) (n = 4)	163 ± 8*	270 ± 31**	118 ± 11**	16 ± 1.6*
	Inulin (5.83 mg/kg)	wild-type (n = 7)	97 ± 9	194 ± 16	447 ± 51	14 ± 1.5
		Oat3(-/-) (n = 6)	92 ± 7	178 ± 17	449 ± 37	13 ± 1.3
Female	PAH (1.430 µg/kg)	wild-type (n = 4)	247 ± 11	503 ± 17	95 ± 5.0	15 ± 0.9
		Oat3(-/-) (n = 5)	241 ± 9.0	477 ± 17	109 ± 5.0	13 ± 0.7
	ES (0.526 µg/kg)	wild-type (n = 4)	45 ± 26	469 ± 85	21 ± 2.0	25 ± 2.0
		Oat3(-/-) (n = 4)	61 ± 17	324 ± 44	27 ± 2.0*	20 ± 1.0*
	PCN G (1.870 µg/kg)	wild-type (n = 4)	184 ± 23	349 ± 25	55 ± 2.8	34 ± 1.7
		Oat3(-/-) (n = 4)	91 ± 23*	209 ± 13**	167 ± 23***	11 ± 1.4***
	Inulin (5.83 mg/kg)	wild-type (n = 8)	100 ± 9.0	199 ± 18	370 ± 39	16 ± 2.0
		Oat3(-/-) (n = 9)	100 ± 4.0	207 ± 11	434 ± 40	14 ± 1.0

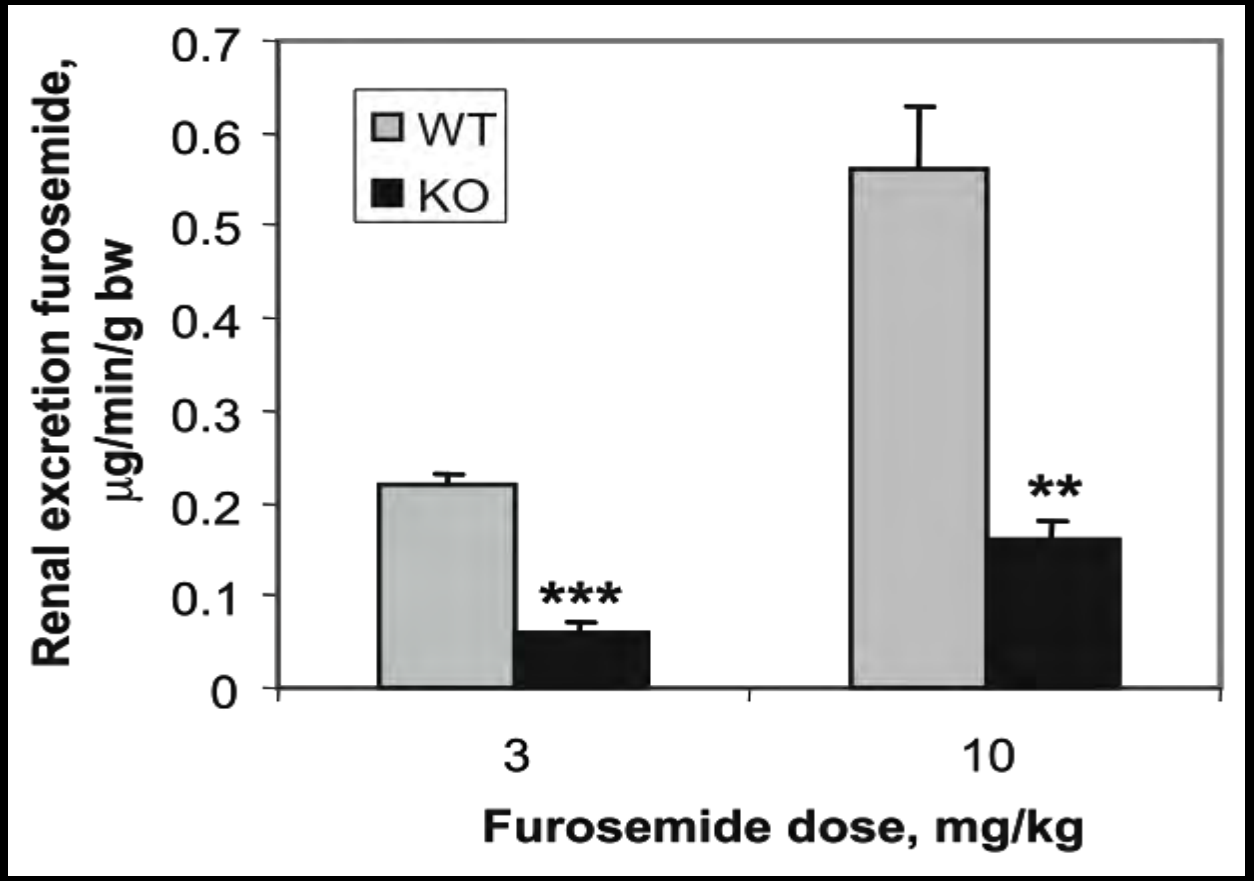
Reduced Diuretic Efficacy in Oat1 Knockout Mice





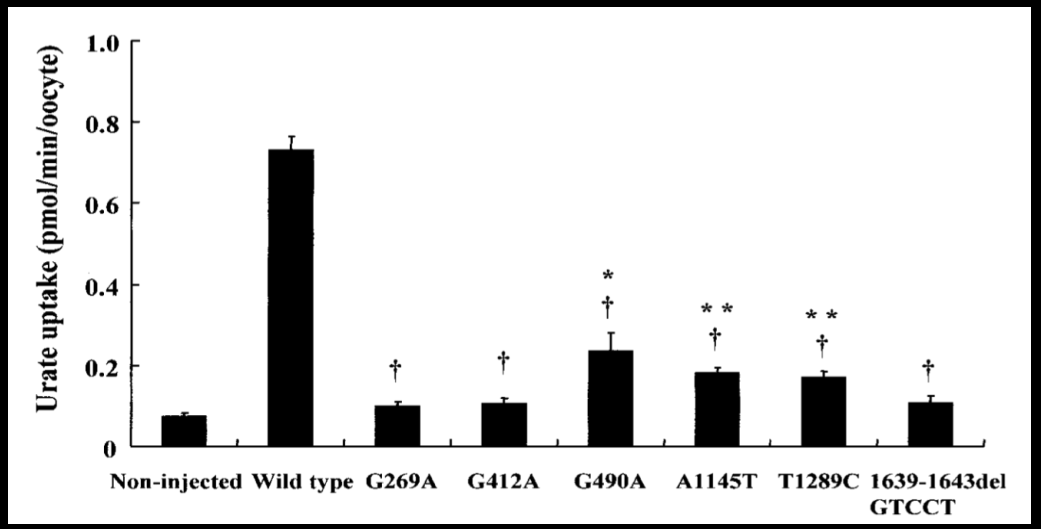
Eraly, et al. *J Biol Chem* 281, 2006

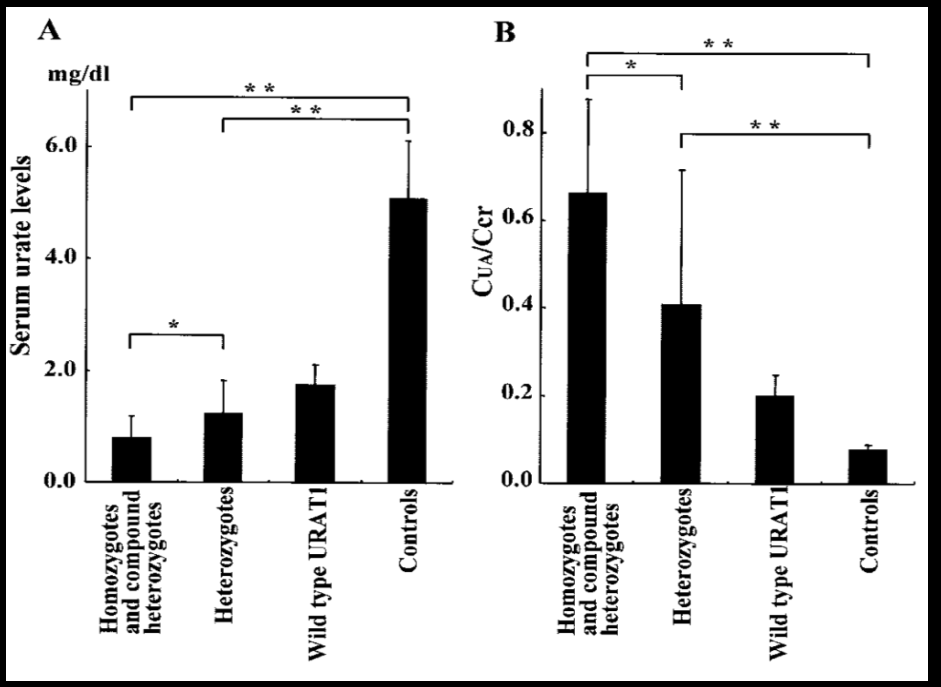
Reduced Excretion in Oat1 Knockout Mice



Eraly, et al. *J Biol Chem* 281, 2006

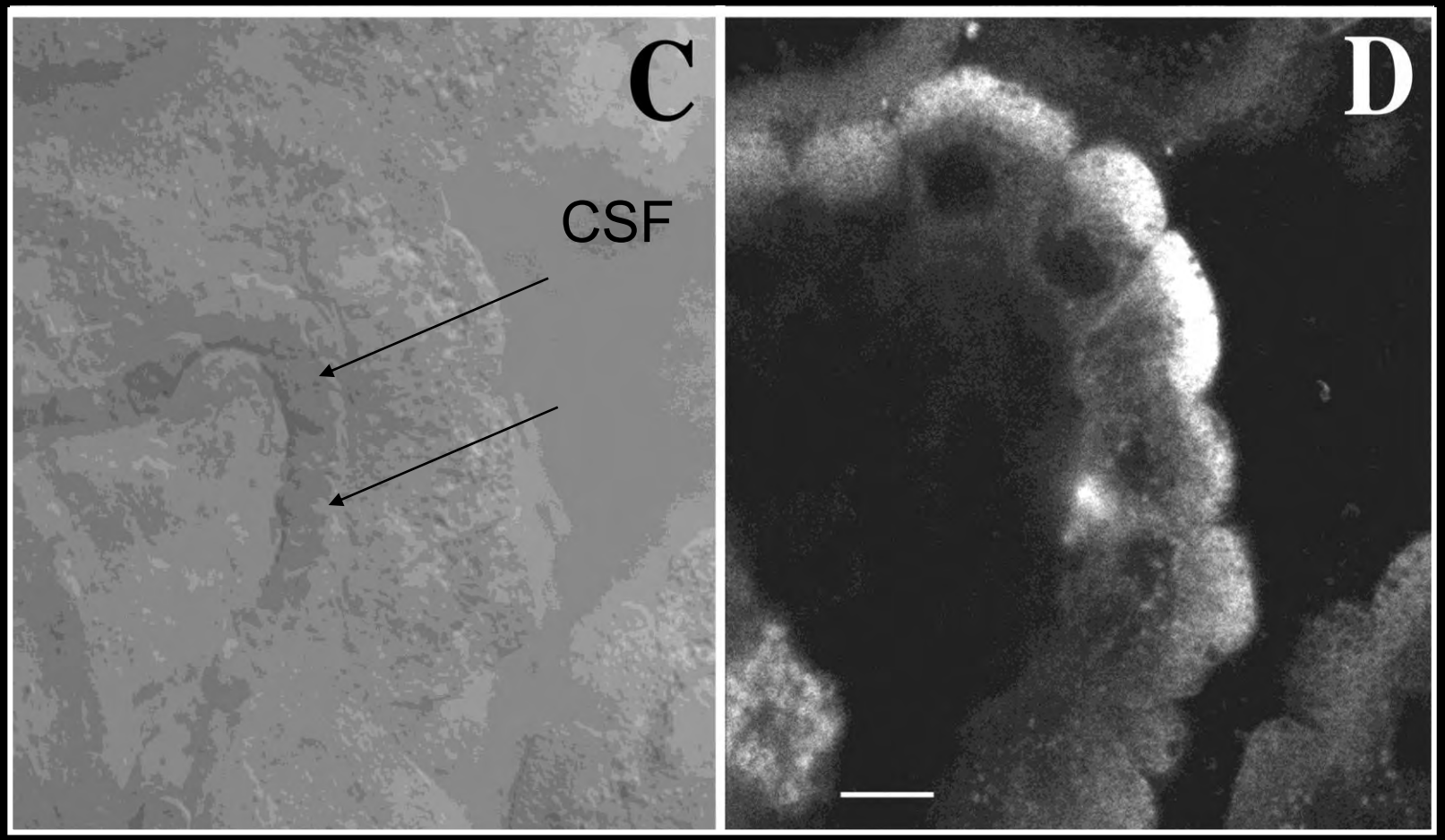
Role of URAT1 in Hereditary Renal Hypouricemia





Ichida et al., *JASN* 15, 2004

Choroid Plexus Morphology



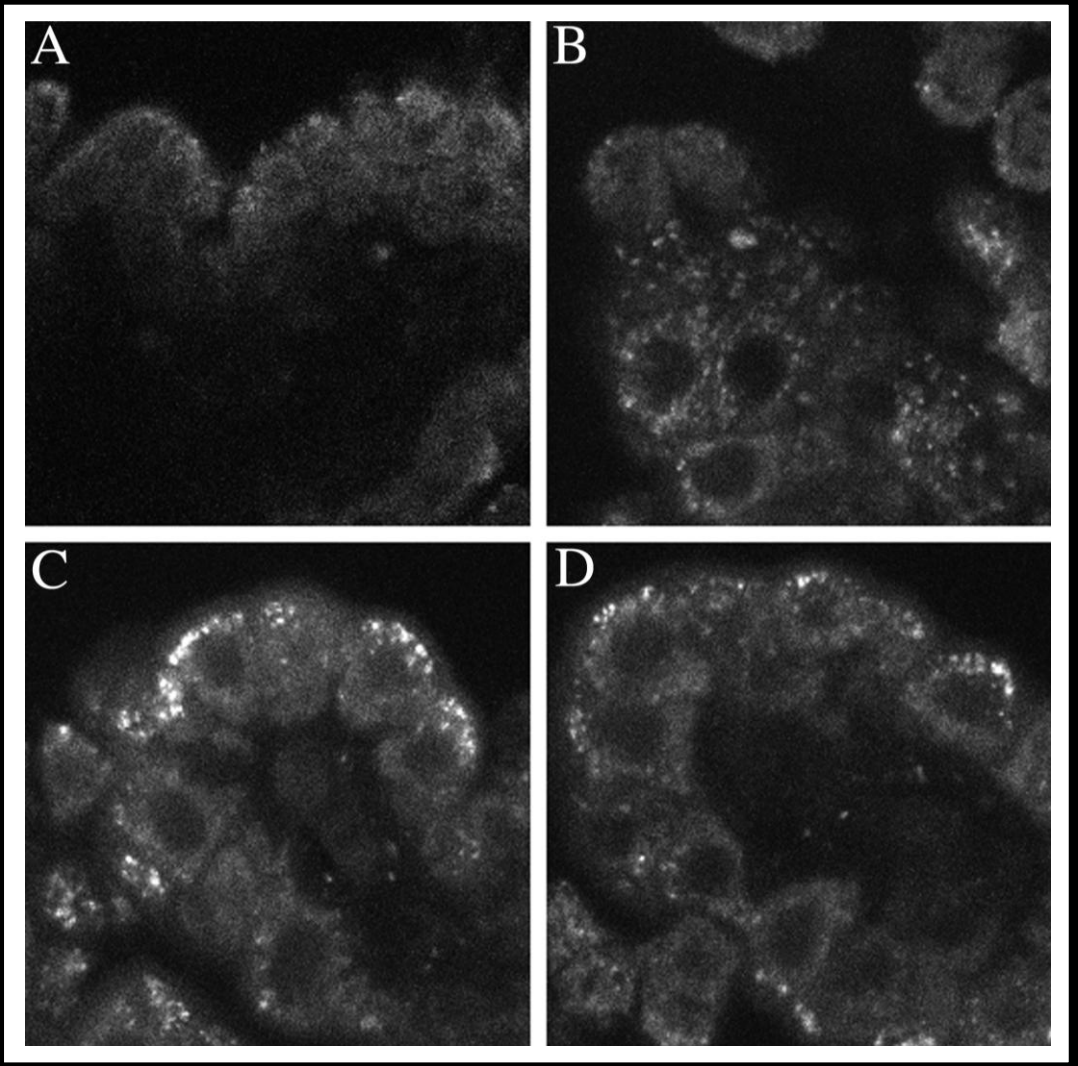
Sweet, D. H., et al. J Biol Chem 276: 41611-41619, 2001

GFP Constructs Expressed in Rat Choroid Plexus

GFP

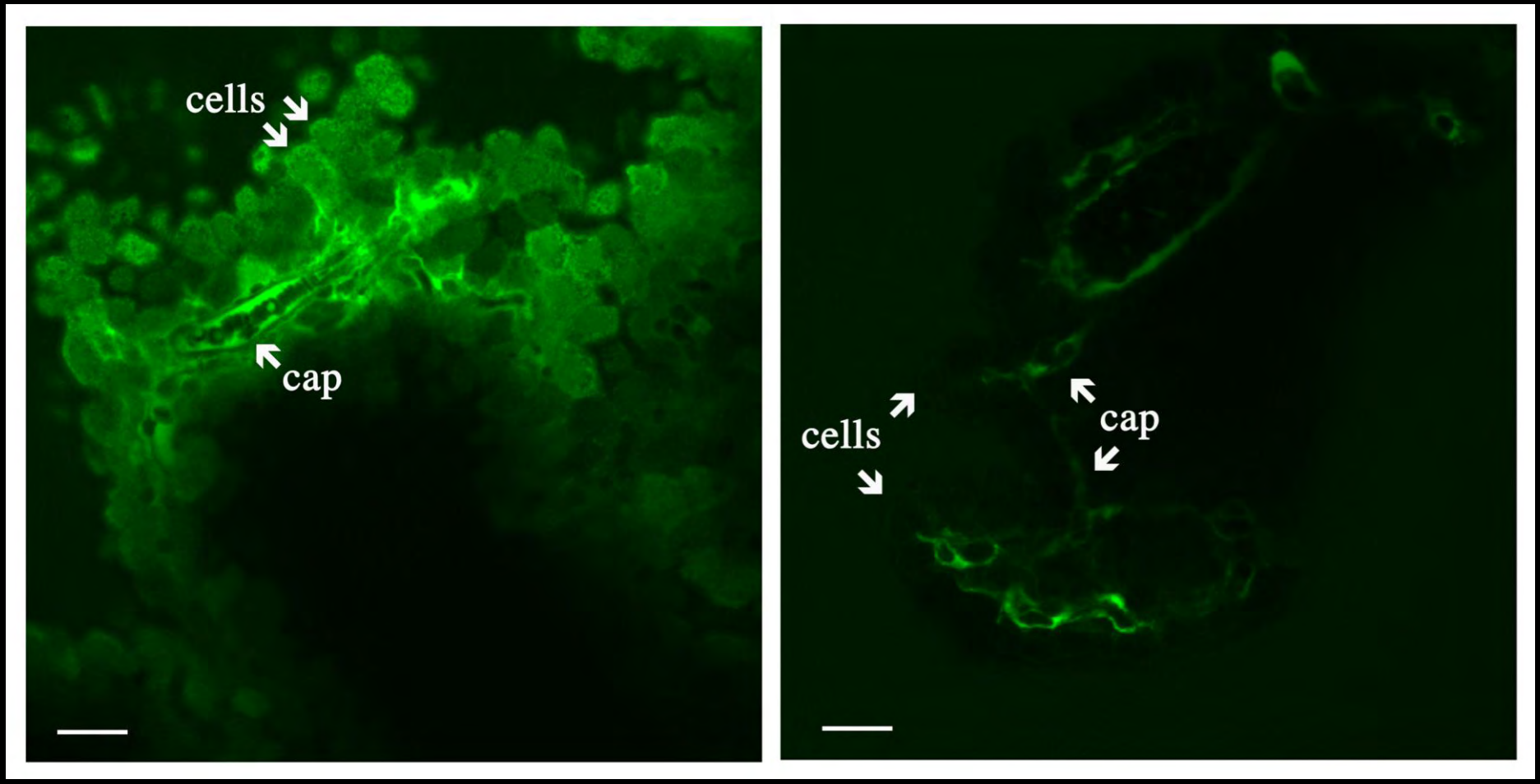
ER-GFP

rOct2-GFP



Sweet, D. H., et al. J Biol Chem 276: 41611-41619, 2001

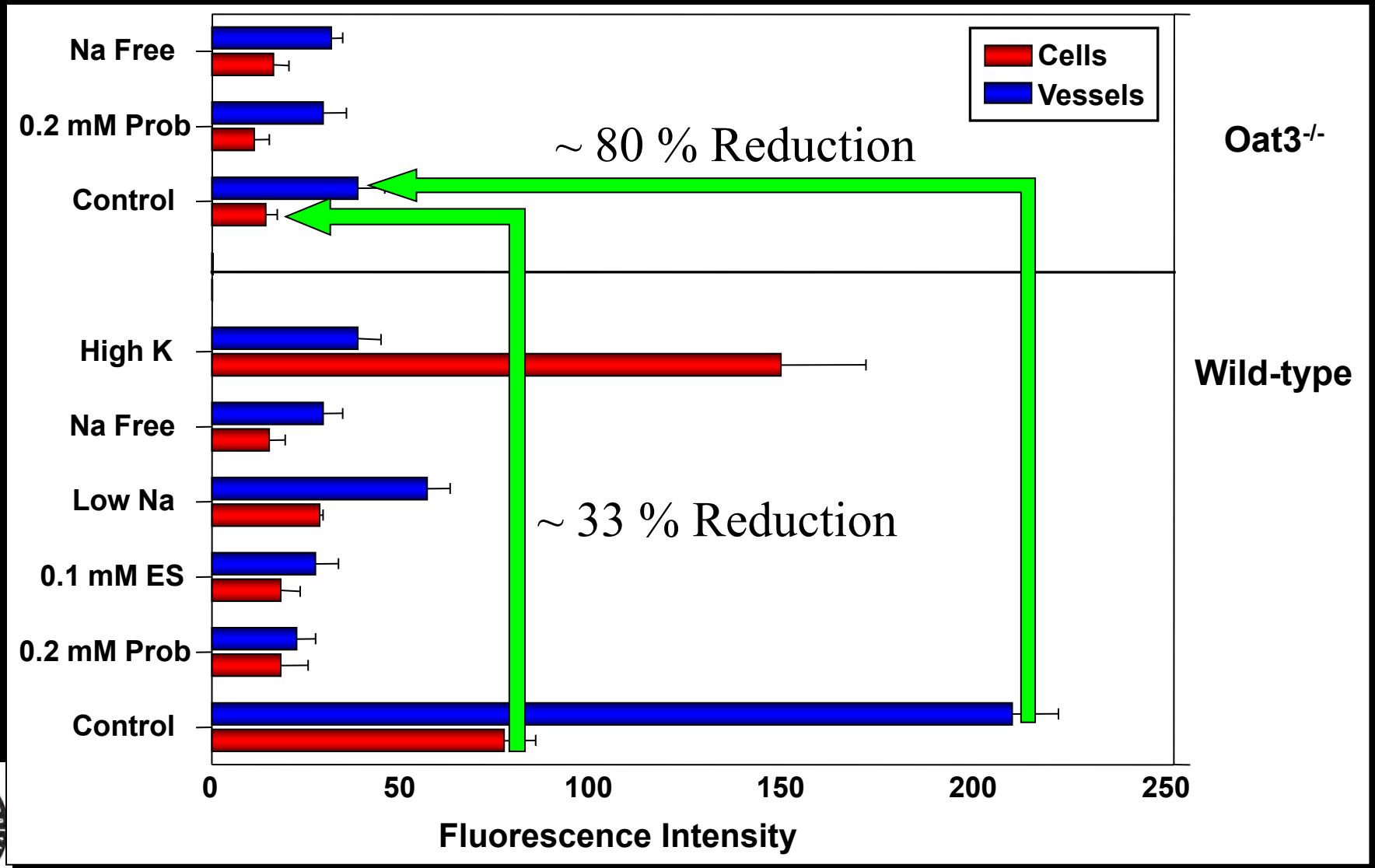
Fluorescein Transport in Murine Choroid Plexus



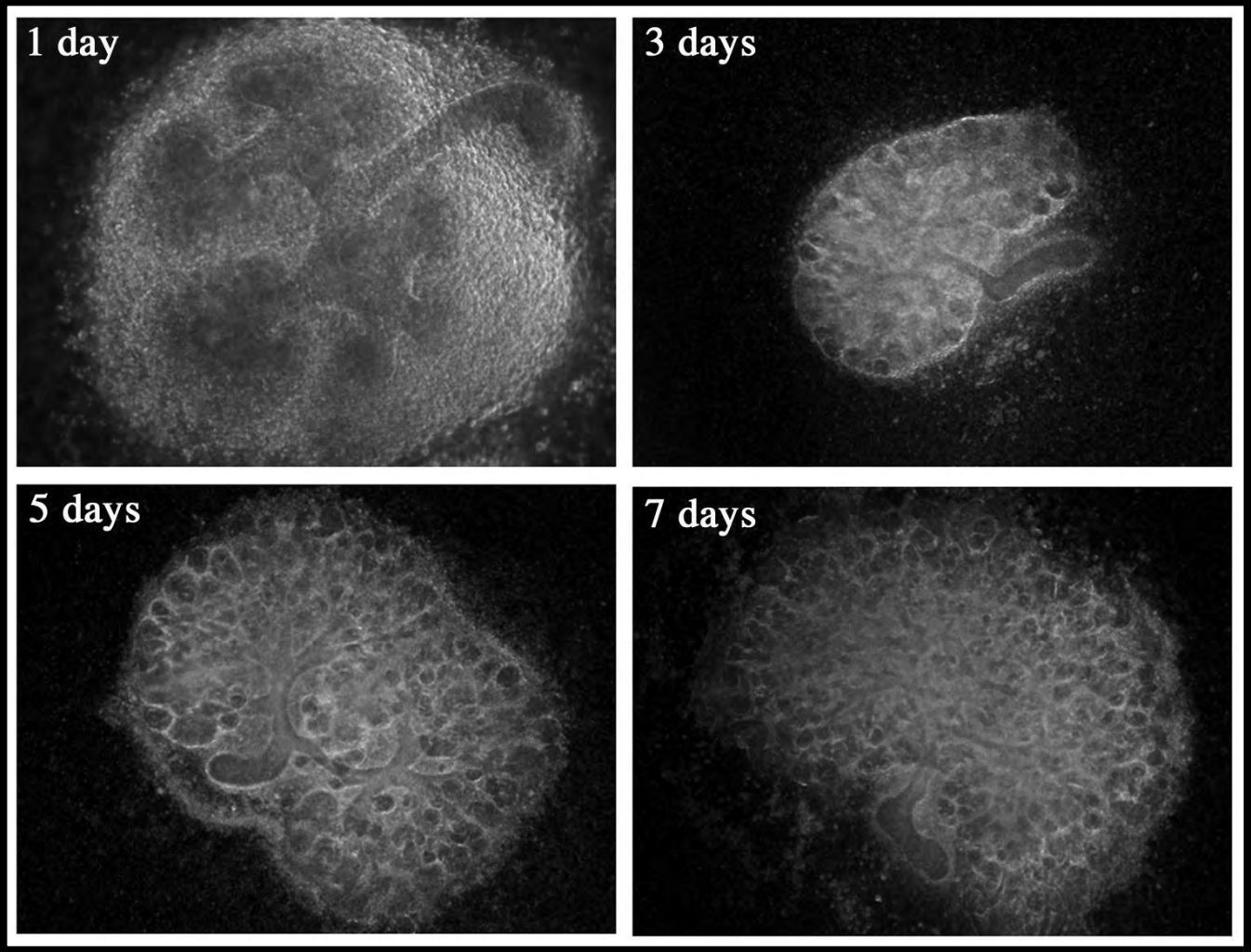
Wild-type

***Oat3*$^{-/-}$**

FL Transport by Wild-type and *Oat3*$^{(-/-)}$ CP



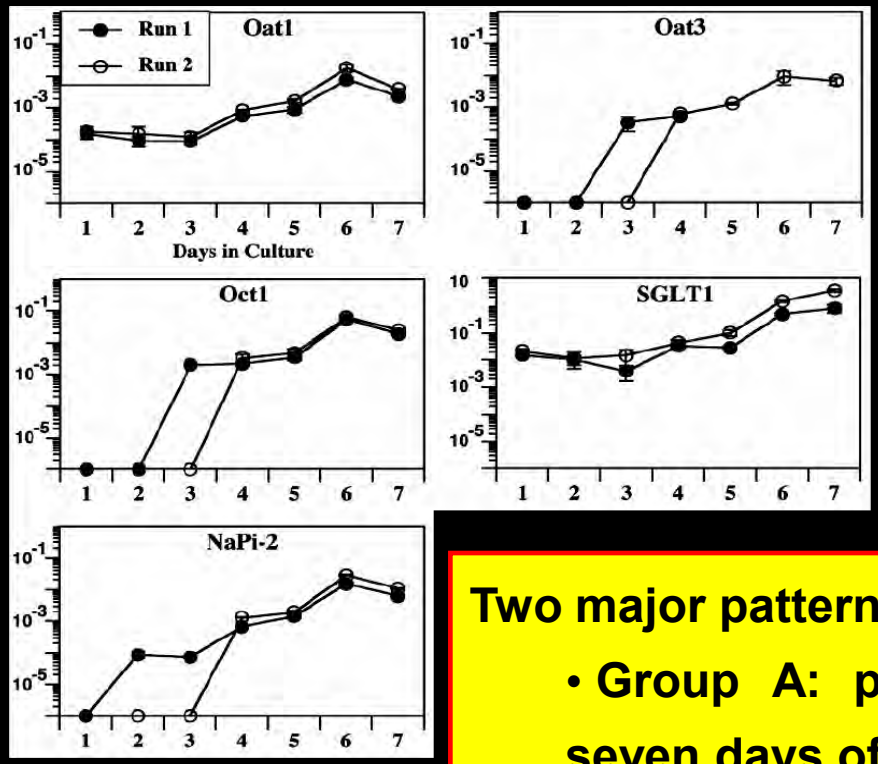
Cultured Kidney Model



Sweet, et al, Kidney International 69: 837-845 (2006)

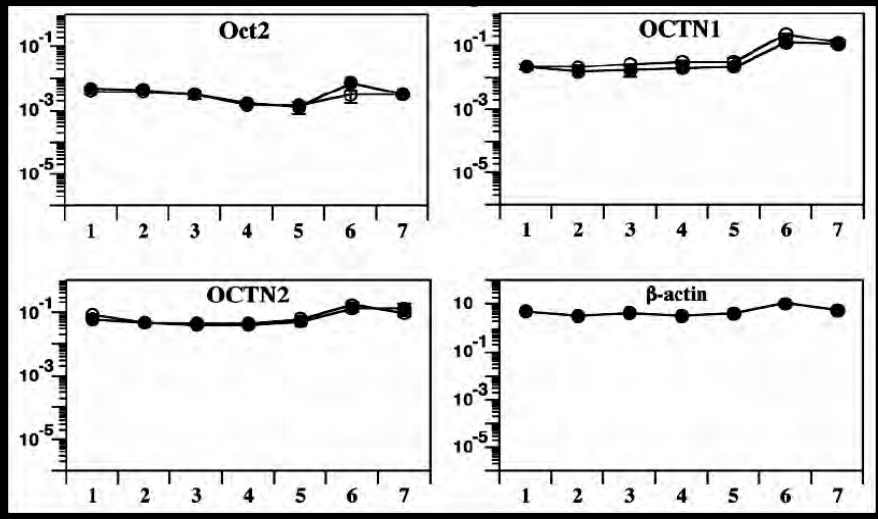
Relative Gene Expression in Cultured Kidneys

Group A



mRNA level Relative to Adult

Group B

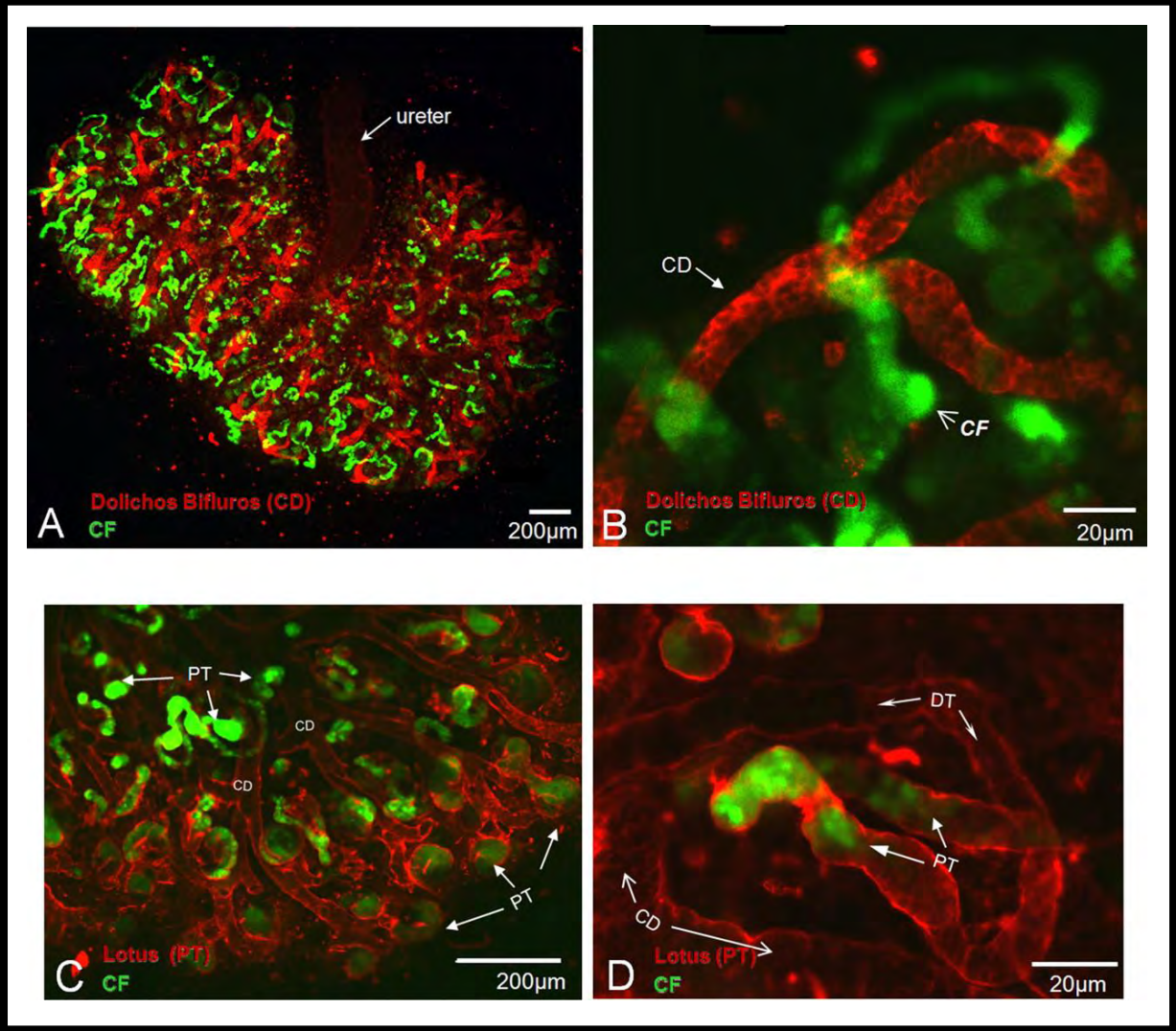


Two major patterns of gene expression:

- **Group A: progressive increase in expression over the seven days of culture**
- **Group B: expression did not substantially change over the duration of culture**

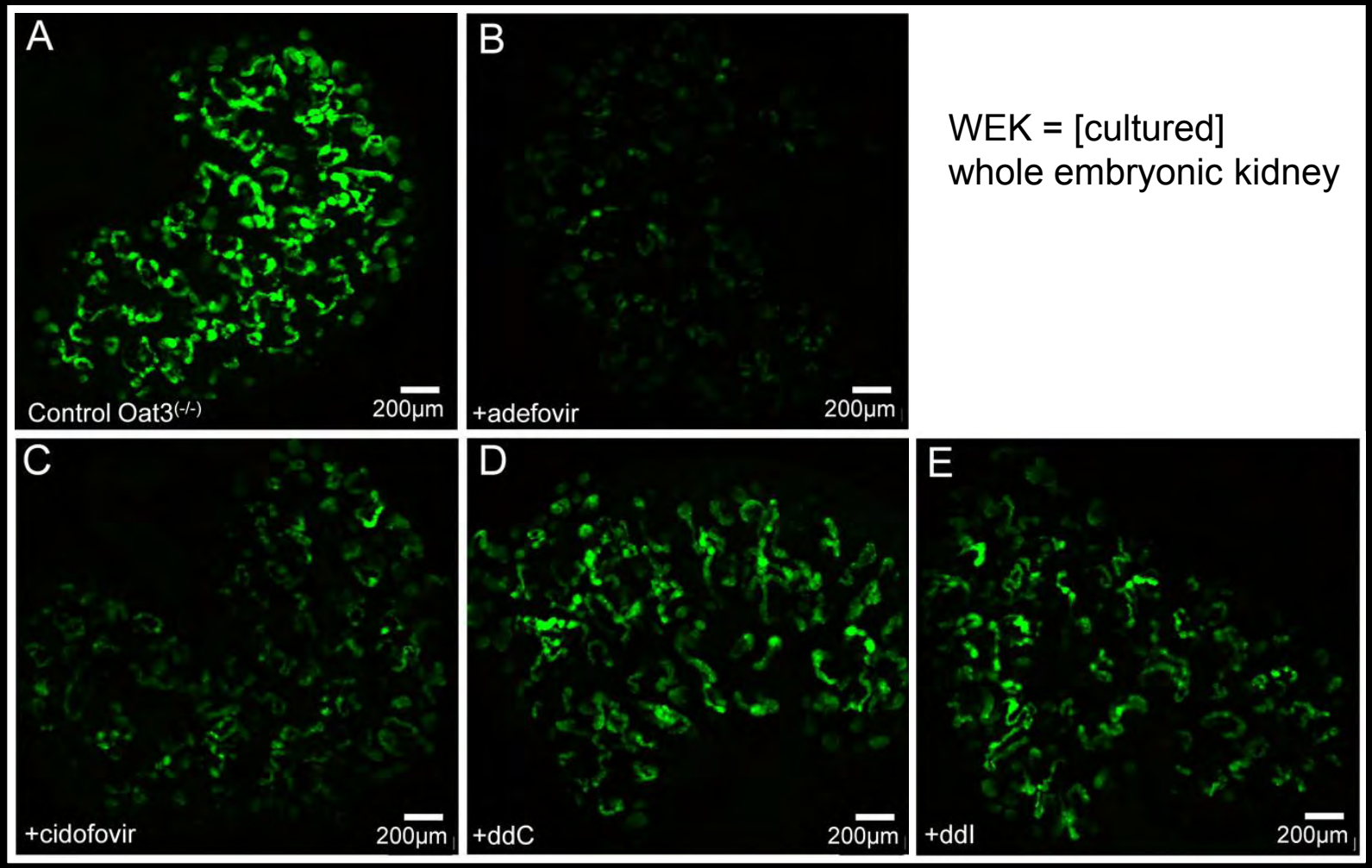
Sweet, et al, Kidney International 69: 837-845 (2006)

Organic Anion Accumulation in Murine Cultured Kidney



Truong, et al, J Biol Chem 283: 8654-8663 (2008)

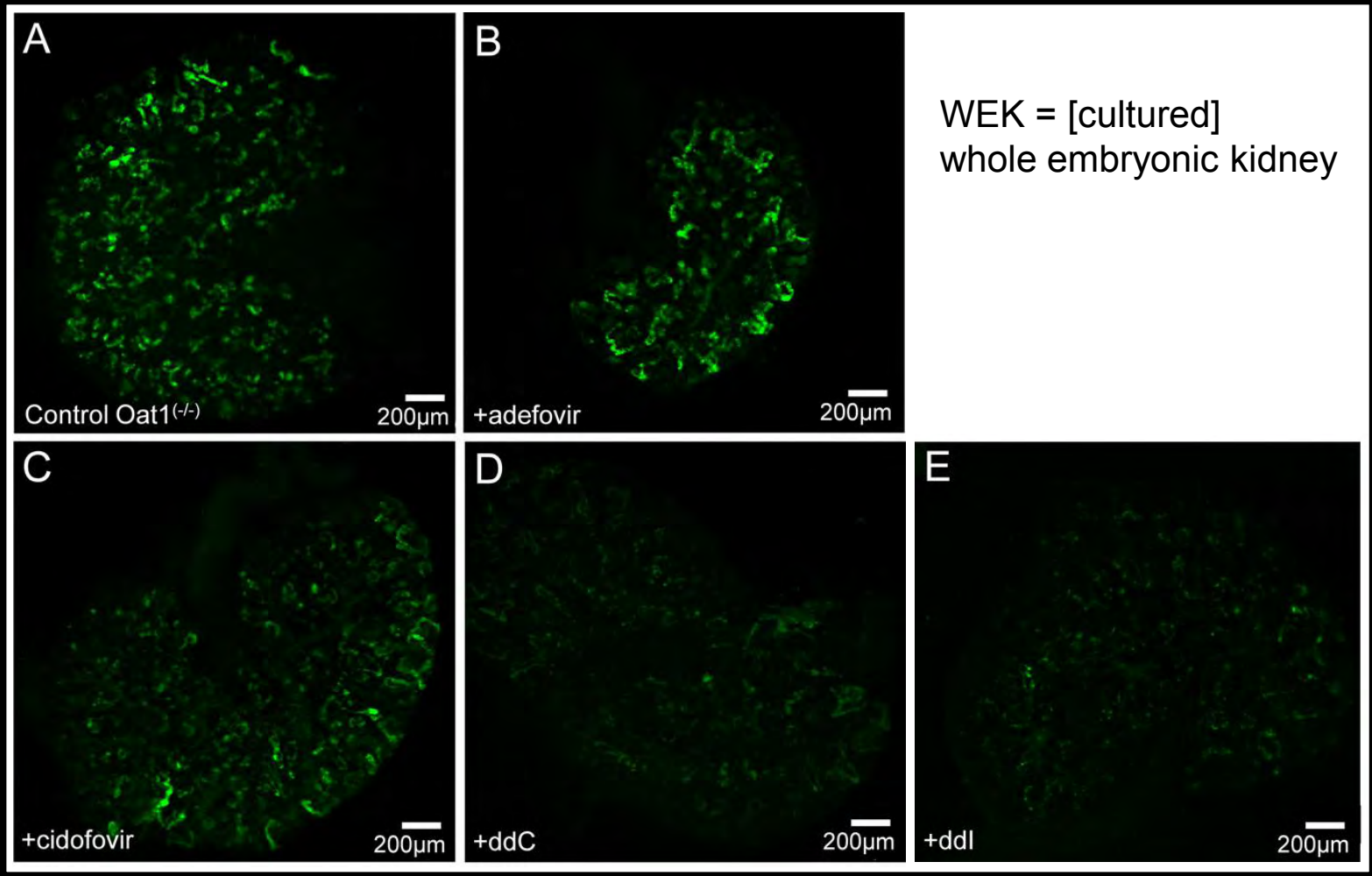
mOat1 Functional Pattern Using mOat3$^{-/-}$ WEK



WEK = [cultured]
whole embryonic kidney

Truong, et al, J Biol Chem 283: 8654-8663 (2008)

mOat3 Functional Pattern Using mOat1$^{-/-}$ WEK

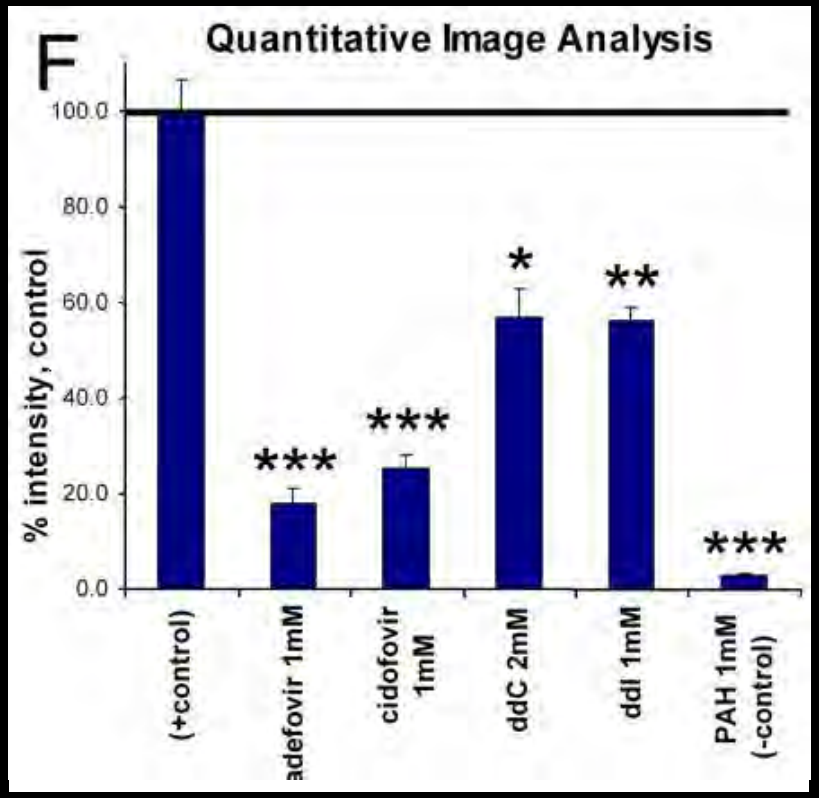


WEK = [cultured]
whole embryonic kidney

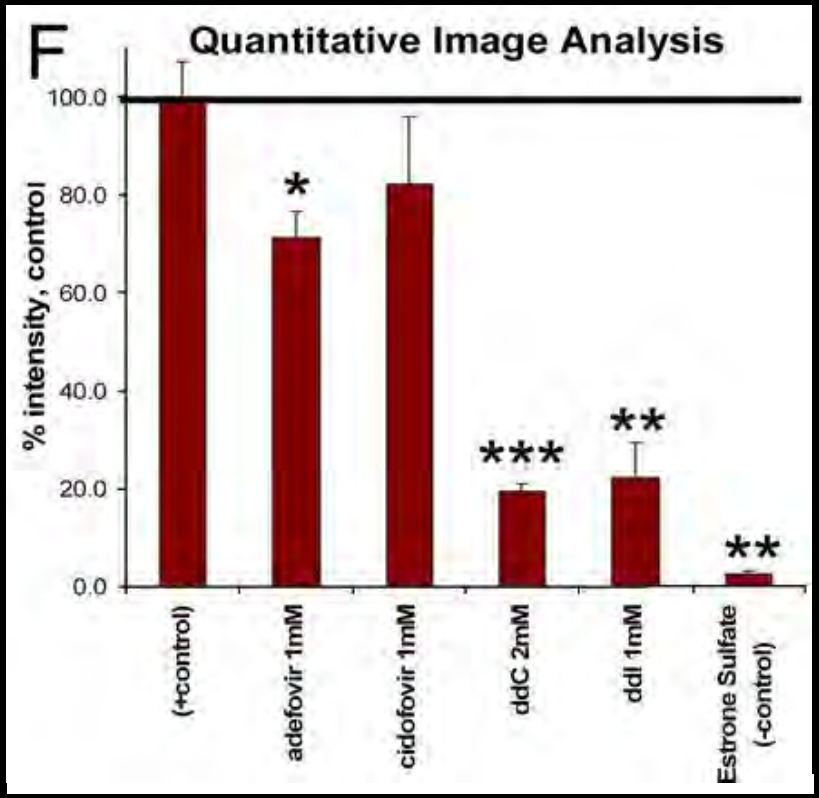
Truong, et al, J Biol Chem 283: 8654-8663 (2008)

Oat3 & Oat1Function in WEK from Knockout Mice

mOat1 function using mOat3$^{-/-}$ WEK

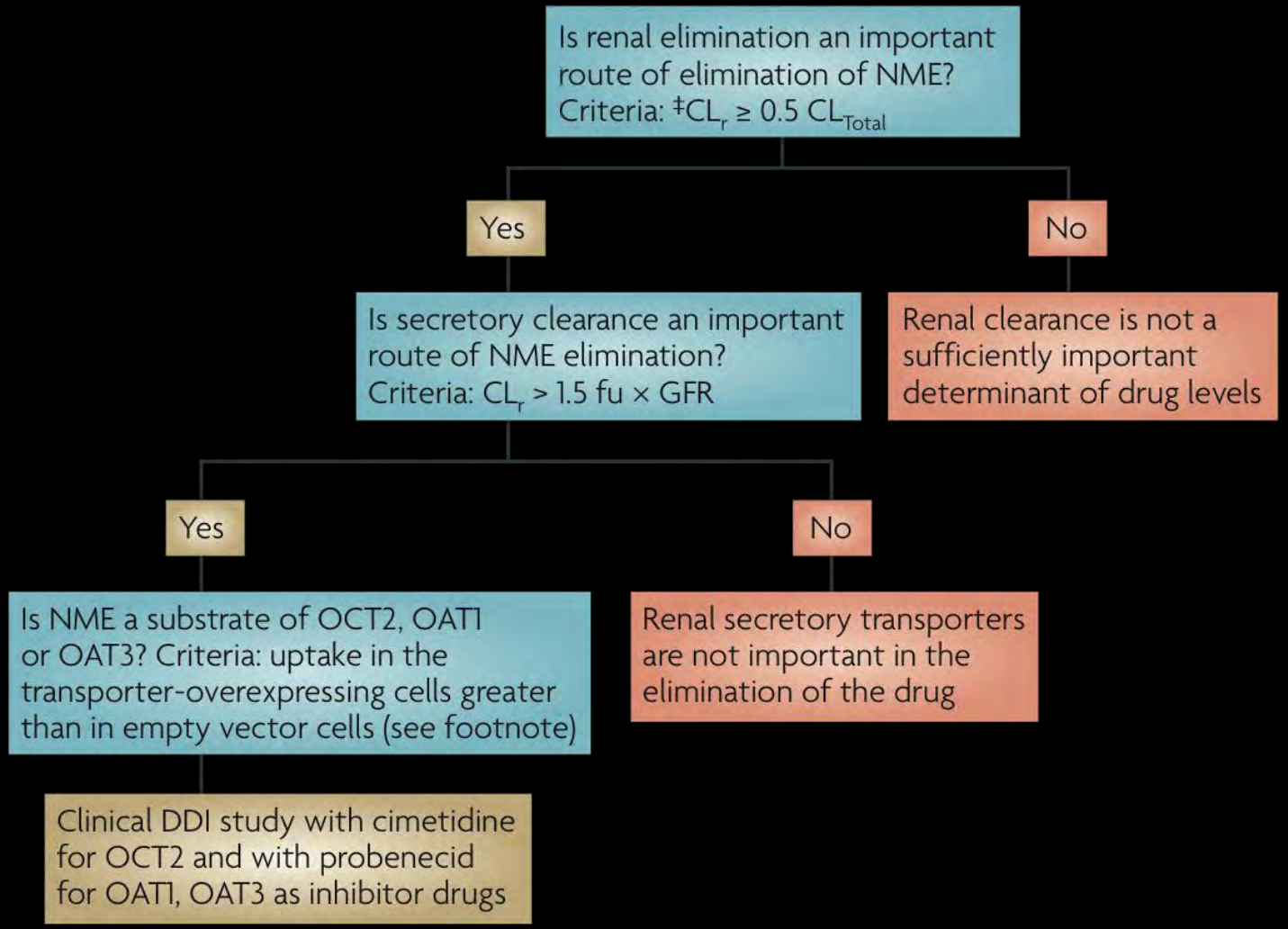


mOat3 function using mOat1$^{-/-}$ WEK

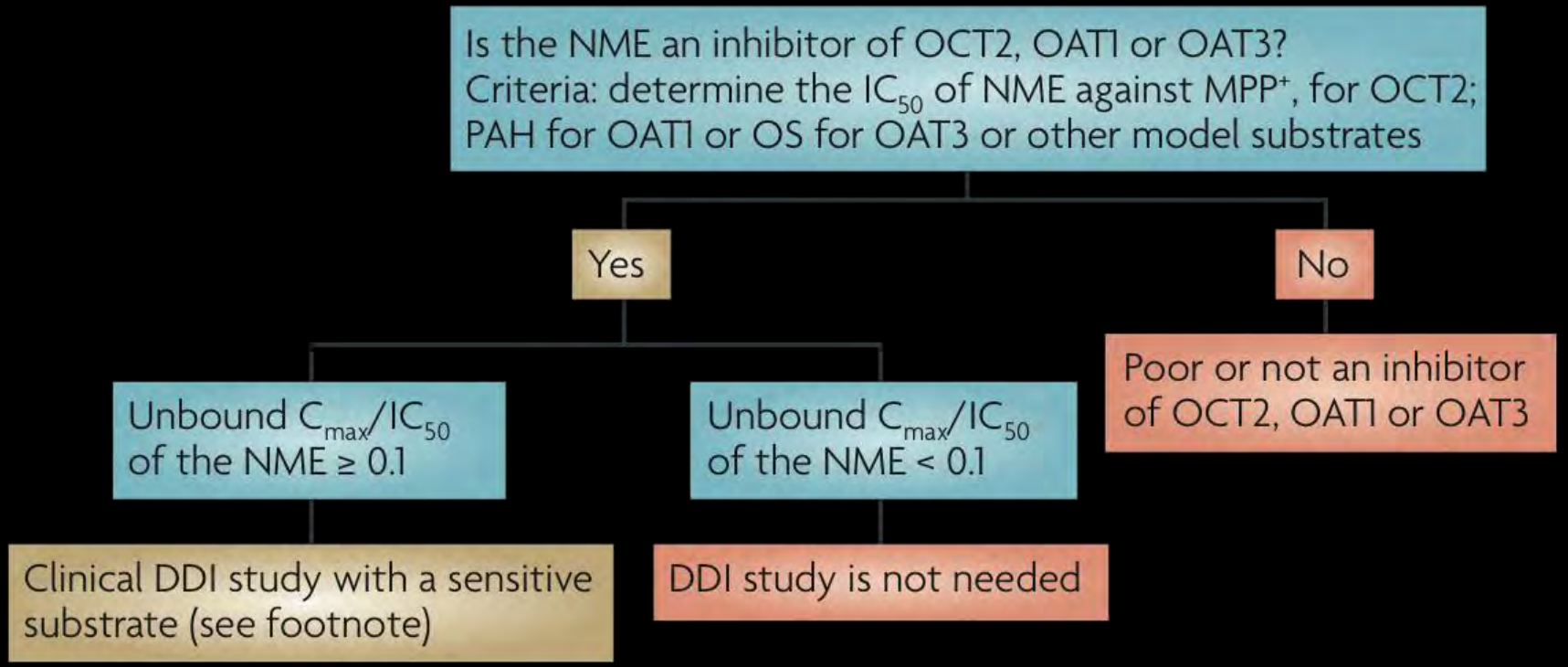


Truong, et al, J Biol Chem 283: 8654-8663 (2008)

Need to Test if an OAT/OCT Substrate?



DDI Study for OAT/OCT Inhibition?



Summary

OATs and OCTs:

- ✓ Impact the disposition and elimination of drugs, endogenous compounds, and xenobiotics
- ✓ This impact on ADME is the combined result of their tissue expression profile, membrane targeting, specificity, and affinity
- ✓ Are a key site for drug-drug interactions
- ✓ Contribute to some disease states
- ✓ May contribute to interindividual variation observed in patient populations

Virginia Commonwealth University
VCU Medical Center

Summary

Oat3 knockout mice revealed:

- ✓ Oat3 is targeted to the basolateral membrane in renal proximal tubules
- ✓ *In vivo* Oat3 does not play a major role in PAH elimination
- ✓ Gender differences in steroid conjugate elimination (M > F)
- ✓ Oat3 is pivotal for penicillin G drug elimination *in vivo*
- ✓ Oat3 is expressed and functional in choroid plexus
- ✓ Oat3 is targeted to the apical membrane and positioned to mediate the efflux of organic anions from CSF to blood

Summary

Ex vivo organ culture model allows:

- ✓ quantitation of OAT/OCT function in intact, polarized tissue with native expression
- ✓ monitoring of uptake and efflux pathways
- ✓ functional assay in transgenic and knockout animals; model SNPs
- ✓ access to organs/tissues at any developmental stage

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