

Nephron Number is Variable and Declines with Age

A Simple Experiment:
Count the number of nephrons at autopsy.

Data: 2-3 fold variation at any age of patient; decline in nephron number with age.

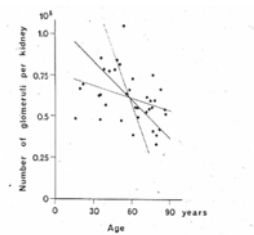


Fig. 2. The number of glomeruli per kidney declines significantly with age, $2^p < 0.001$; $r = -0.35$. Two possible explanations exist. Normal human beings may lose glomeruli with age, or more likely, older human beings were born with fewer glomeruli than younger human beings. Apparently, the number of glomeruli starts to decline about the age of 60 years.

Nephron Number in Patients with Primary Hypertension

Gunhild Keller, M.D., Gisela Zimmer, M.D., Gerhard Mall, M.D., Eberhard Ritz, M.D., and Kerstin Amann, M.D.

Background A diminished number of nephrons has been proposed as one of the factors contributing to the development of primary hypertension.

Methods To test this hypothesis, we used a three-dimensional stereologic method to compare the number and volume of glomeruli in 10 middle-aged white patients (age range, 35 to 59 years) with a history of primary hypertension or left ventricular hypertrophy (or both) and renal arteriolar lesions with the number and volume in 10 normotensive subjects matched for sex, age, height, and weight. All 20 subjects had died in accidents.

Results Patients with hypertension had significantly fewer glomeruli per kidney than matched normotensive controls (median, 702,373 vs. 1,429,200). Patients with hypertension also had a significantly greater glomerular volume than did the controls (median, $6.50 \times 10^{-4} \text{ mm}^3$ vs. $2.79 \times 10^{-4} \text{ mm}^3$; $P < 0.001$) but very few obsolescent glomeruli (5.5%).

Conclusions The data support the hypothesis that the number of nephrons is reduced in white patients with primary hypertension.

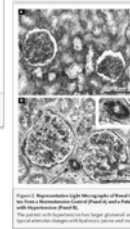
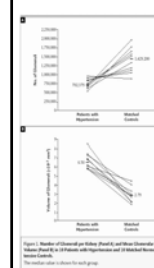
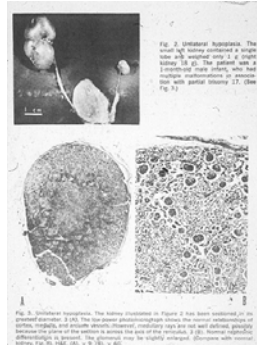


Figure 2. Representative light micrographs of renal tissue. A, Normal glomerulus. B, Glomerulus with increased mesangial matrix and some sclerosis. C, Glomerulus with more advanced sclerosis and obsolescence.

Unilateral Renal Hypoplasia

1. Severe Defect in Nephron Number
Unilateral hypoplasia= 1% of the population. Perhaps asymmetries seen by ultrasound of our patients in the hospital are more subtle forms of unilateral hypoplasia.
2. **Bilateral disease**= functional for 5-10yrs; then constitutes 20% of the juvenile population on dialysis.



1. Defect in nephrogenesis produces abnormal cells- a change in the developmental program.
2. Usually diffuse and hence incompatible with normal renal function

Renal Dysplasia

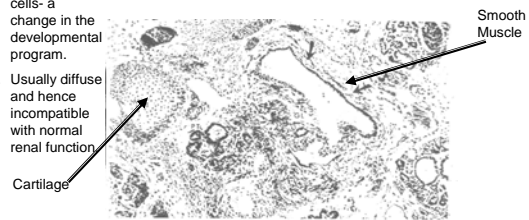


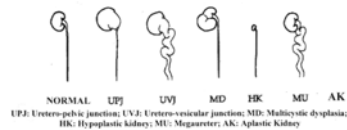
Fig. 4. Renal dysplasia. Cystic structures are abnormal and in places. A section of tubule (PT) lined by poorly differentiated 'epithelial' cells and a central duct is surrounded by a non-renal structure (cartilage). The glomerulus (GL) has an irregular appearance. Mesangial cysts (MC) represent abnormally differentiated mesangial tissue. (H&E, $\times 150$ phase-contrast).

"Diffuse cystic dysplasia"-- little normal
"Multicystic dysplastic"--cords of normal

Hypoplasia and Dysplasia Account for 50% of Abdominal Masses of the Newborn (Prevalence 0.5% of all Pregnancies)

Both Hypoplasia and Dysplasia are associated with urologic abnormalities, but the cause and effect is not clear:

1. Uretero-pelvic junction UPJ obstruction.
2. Uretero-vesicular junction UVJ obstruction.
3. Mega-ureter.



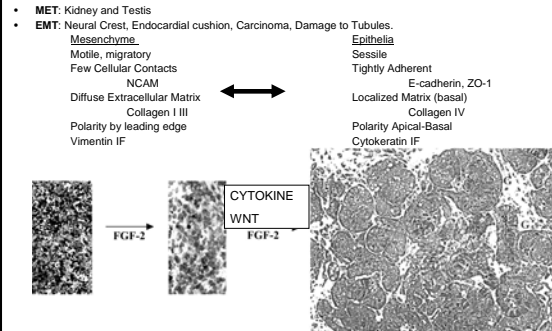
Kidney Development<<>>Urological Development

- How to make a nephron
- How to make 10^6 nephrons.
- How to connect nephrons from kidney to bladder.
- How nephron formation is related to hypoplasia and dysplasia and how these are related to abnormalities of the urinary track.

Concepts

- Lineage marker
- Epiboly and experiments that investigated the “duct”.
- The plasticity of the duct systems.
- Induction and Reciprocal Induction
- Mesenchymal to epithelial conversion
- Monopodial & dipodial branching
- Stephen’s Hypothesis and the “shared molecule hypothesis”

The Central Mechanism of Kidney Development is the Conversion of Mesenchyme into Epithelia



The Intermediate Mesoderm

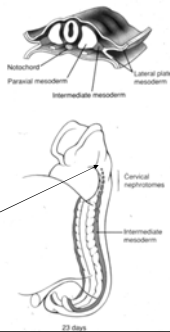
“TheCord”

At the end of gastrulation a sheet of cells runs the length of the embryo- “the mesoderm”.

The mesoderm segregates into three compartments; the middle one is called the “intermediate mesoderm”

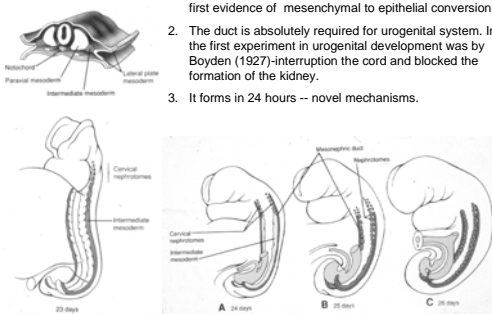
The extraordinary characteristic of these cells is that they can convert into epithelial cells.

Pronephros Epithelializes:
 At the cranial end of the cord a series of vesicles form the pronephros. The mechanism is not known in mammals and these vesicles will degenerate.



Wolffian Duct Forms in One Day

1. Epithelialization of the pronephros and then the duct are the first evidence of mesenchymal to epithelial conversion.
2. The duct is absolutely required for urogenital system. In fact, the first experiment in urogenital development was by Boyden (1927)-interruption the cord and blocked the formation of the kidney.
3. It forms in 24 hours -- novel mechanisms.



How Does the Wolffian Duct Form So Quickly?

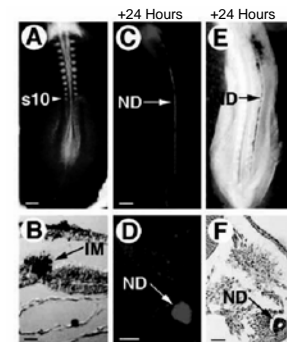
- **H:** A tubule forms in the cranial part of the cord and then the epithelial cells proliferate and migrate caudally.
Exp: Coherent growth of an epithelial cord; very high replication rate in these cells.
- **H:** A wave of Mesenchymal to Epithelial Conversion.
 1. Cells could incorporate along the length of the cord as they convert into epithelia.
Exp: the tubule to be a mosaic of cells.
 2. Cord is formed and then converts in situ in a two step process.
Exp: We could dissociate cord formation and epithelialization.

To determine the mechanism of duct formation Herzlinger introduced a lineage marker into different sites of the intermediate mesoderm.

Lineage marker is 1. a label that is maintained by the progeny of a cell, no matter what they become ie not subject to variation in gene expression. 2. does not spread from cell to cell.

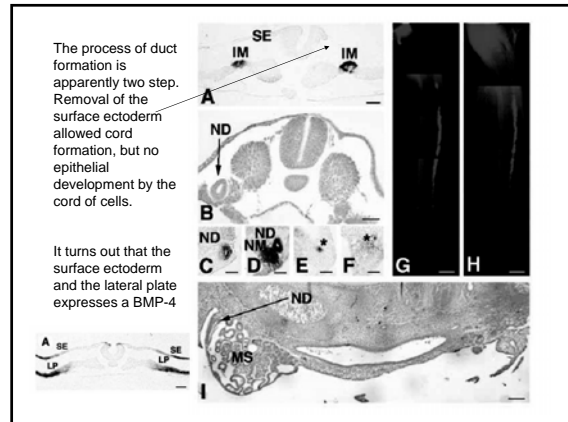
She found that a stream of labeled cells appeared within 24 hours of marking one spot at s10. This stream was the nephric duct-we see it in cross section.

This experiment indicates that migration is involved but its too fast for cell replication to be the driving force. Also it shows that cells are not being incorporated from an external source, because otherwise it would appear as a mosaic.



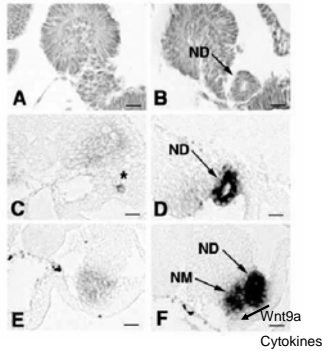
How Does the Wolffian Duct Form So Quickly?

- **H:** A tubule forms in the cranial part of the cord and then the epithelial cells proliferate and migrate caudally.
Exp: Coherent growth of an epithelial cord; very high replication rate in these cells.
- **H:** A wave of Mesenchymal to Epithelial Conversion.
1. Cells could incorporate along the length of the cord as they convert into epithelia.
Exp: the tubule to be a mosaic of cells.
2. Cord is formed and then converts in situ in a two step process.
Exp: We could dissociate cord formation and epithelialization.



Rescue Experiment: Add beads soaked in albumen or BMP-4.

1. Wolffian duct has a stem cell that generates migrating cells. The migration is 'special' - it may involve rolling of cells over each other creating something like epiboly
2. Migration of cord cells and then their epithelialization are independent.
3. Another finding is the activation of Pax-2 transcription in the surrounding mesenchyme. Pax-2 is a transcription factor that is necessary for the next step. Note that it is the epithelialized duct that triggers Pax-2, not the cord.

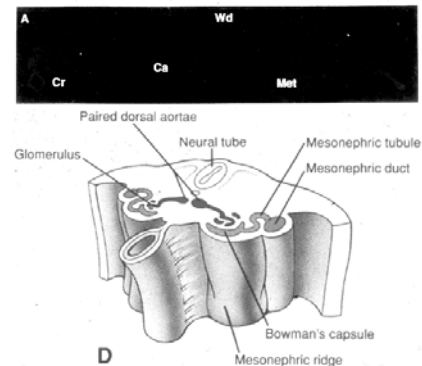


Concepts

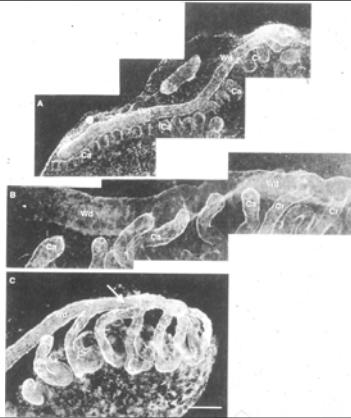
- *Lineage marker*
- *Epiboly and experiments that investigated the "duct".*
- The plasticity of the duct systems.
- Mesenchymal to epithelial conversion
- Induction and Reciprocal Induction
- Monopodial & dipodial branching
- Stephen's Hypothesis and the "shared molecule hypothesis"

Wolffian Duct>>?

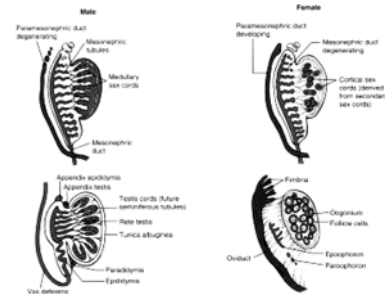
- Changes the surrounding duct cells to express Pax-2.
- Pax-2 is required for all subsequent steps.
- As the Wolffian duct advances caudally, nephrons appear on either side of the duct. The nephrons form perpendicular to the cord. The are called 'mesonephric tubules'. 20-30 tubules form. It is functional in amphibia and fish, but in mammals only the cranial 4-6 attach to the Wolffian duct, and the rest will degenerate.



We would have never talked about these mesonephric tubules at all, except in males they never go away entirely! They are hijacked by the testis. The cranial ones find the testis and form the ductuli efferentes of the epididymis, and the Wolffian duct becomes the conduit for sperm—the vas deferens.



In females the mesonephric tubules degenerate nearly entirely leaving behind the epoophoron, a group of cysts.



Concepts

- *Lineage marker*
- *Epiboly and experiments that investigated the "duct".*
- *The plasticity of the duct systems.*
- Mesenchymal to epithelial conversion
- Induction and Reciprocal Induction
- Monopodial & dipodial branching
- Stephen's Hypothesis and the "shared molecule hypothesis"

The Wolffian Duct >> Pax-2+ Intermediate Mesenchyme >> Mesonephros >> Metanephros



Just before the cloaca the UB forms as a bud and then a branch and it invades the caudal part of the cord of mesenchyme, to form the kidney.

Gruenwald in 1940 and Grobstein in 1953.

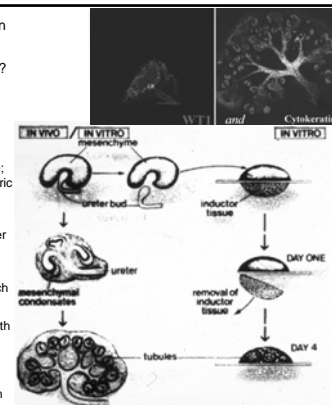
What does this experiment show?

>Sufficiency

>Lineage

>Induction

1. Nephrons come from mesenchyme; Collecting ducts come from the ureteric bud.
2. The mesenchyme is incompetent without the ureteric bud or some other exogenous tissue.
3. The exogenous tissue is needed only 24 hours; it throws a switch. Such a switch is called 'induction'.
4. The ureteric bud is incompetent without the mesenchyme, i.e. reciprocal induction.
5. This is why Boyden stopped renal development by blocking the Wolffian duct.



Reciprocal Induction

- Mutual Dependency
- Implies that factors made in Mesenchyme>>Ureteric bud>>Mesenchyme
- Hence any interruption of the signaling between these compartments must affect both compartments.
- Determination of targeting and downstream signaling is complex because a factor may come from the mesenchyme to instruct the ureteric bud to synthesize a factor important in the mesenchyme.

Reciprocal Induction

- Ureteric Bud>Mesenchyme
 - Wnt6, Wnt9a, Wnt-11, FGF-2,9,18, TIMP, IL-6 Cytokines, BMP-7.
- Mesenchyme>Ureteric Bud
 - GDNF, FGF-7, pleiotrophin, BMP-4, HGF, amphiregulin.
- Stroma>Ureteric Bud
 - RA dependent factor, BMP-4, BMP-6.
- Ureteric Bud>Stroma
 - Unknown signal.
- Mesenchyme>Stroma
 - BMP-7.
- Stroma>Mesenchyme
 - Unknown Signal.

How to Make a Nephron

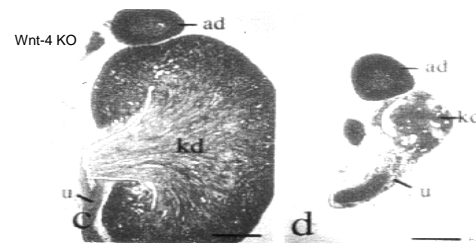
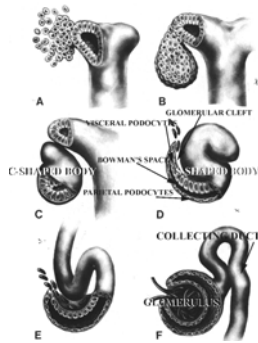
Non-condensed mesenchyme

Condensed mesenchyme

Epithelial Conversion

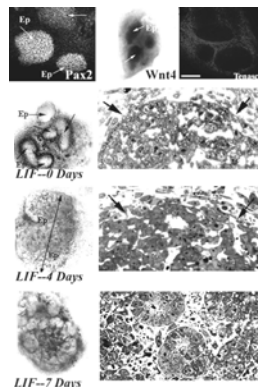
Segmentation

Fusion



UB Produces FGF, Cytokines and Wnts.

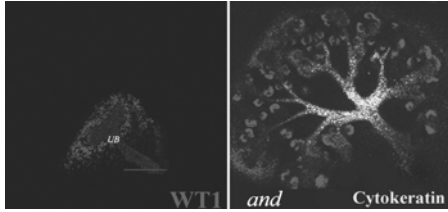
LIF +FGF-2 Acts on "Condensed Mesenchyme"



Concepts

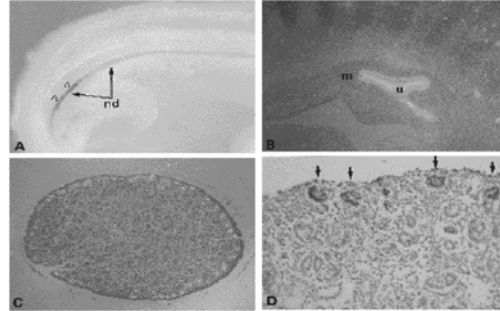
- Lineage marker
- Epiboly and experiments that investigated the "duct".
- The plasticity of the duct systems.
- Mesenchymal to epithelial conversion
- Induction and Reciprocal Induction
- Monopodial & dipodial branching
- Stephen's Hypothesis and the "shared molecule hypothesis"

How to Make a Collecting Duct



At the tips of the ureteric bud is a receptor tyrosine kinase called RET

RET



F Costantini

KO of Ret >> No Ureteric Bud >> First Steps of Mesenchymal Development >> No Kidney

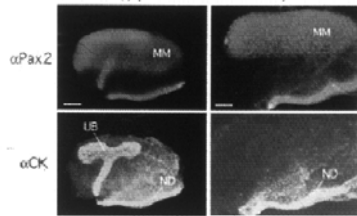
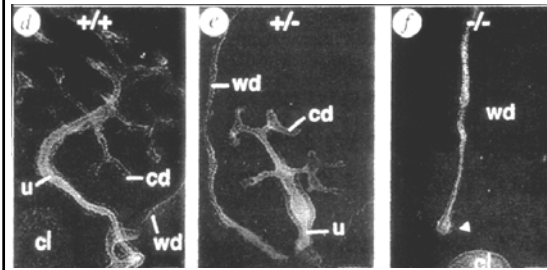


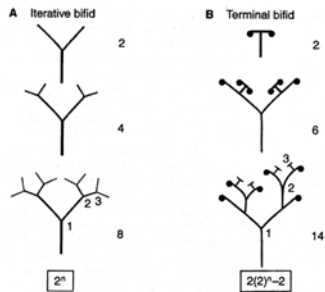
Fig. 1. Expression of Pax2 in E11.5 metanephric mesenchyme. Whole-mount antibody staining with antibodies against Pax2 (red) and pan-cytokeratin (green) in wild-type (+/+) and *Ret*^{-/-} mutants as indicated. Pax2 protein is localized to the nephric duct (ND), ureteric bud (UB) and metanephric mesenchyme (MM) in wild-type E11.5 kidneys. Anti-pan-cytokeratin stains the epithelium of the nephric duct and ureteric bud only. Note the expression of Pax2 in the mesenchyme of *Ret* mutants despite the absence of any ureteric bud. Scale bars: 80 μm.

Dressler

Ligand for Ret= Glial Cell Line Derived Neuronotrophic Factor



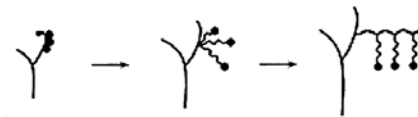
Terminal Bifid Branching Occurs 15 times and Produces 65,534 branches



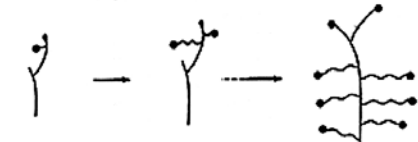
Al-Awqati

Whereas GDNF and FGF-7 may control the initial stages of branching—what controls the complexity of Terminal bifid branching?

A Arcade

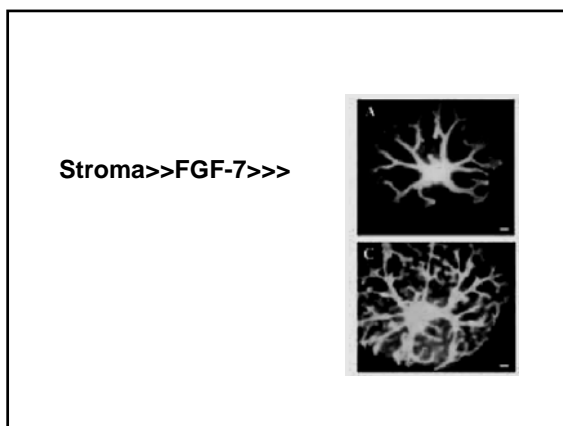
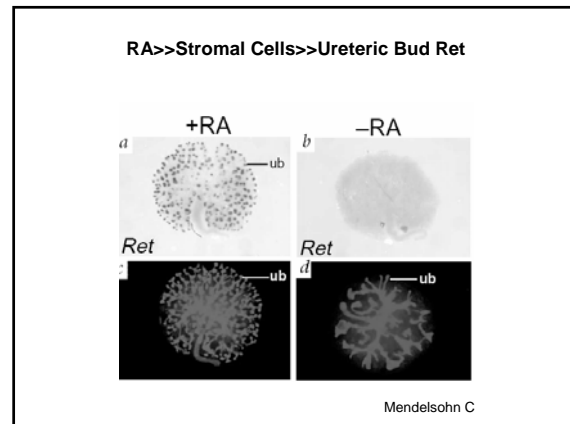
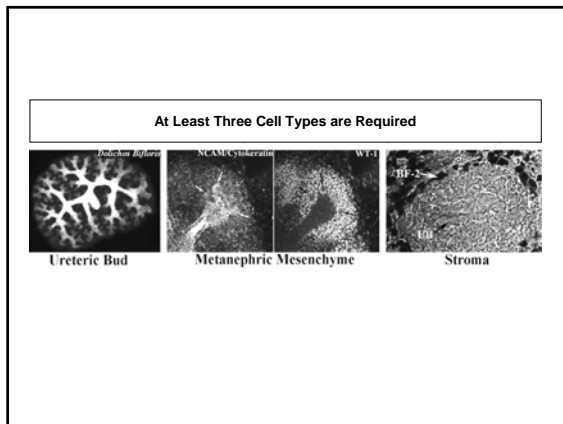


B Cortical lateral system



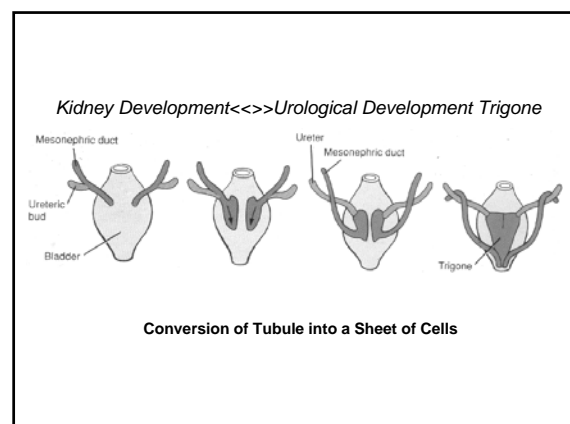
Group Induction—Arcades—5 nephrons

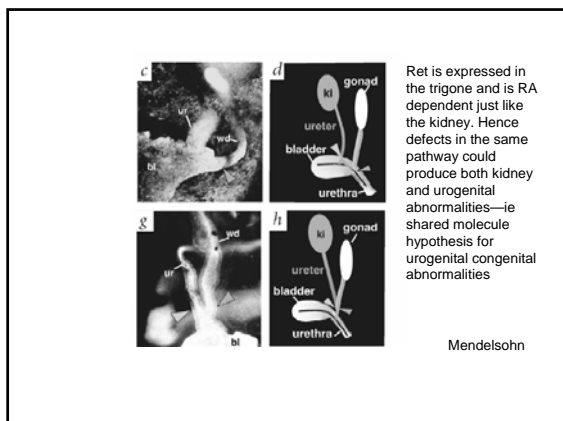
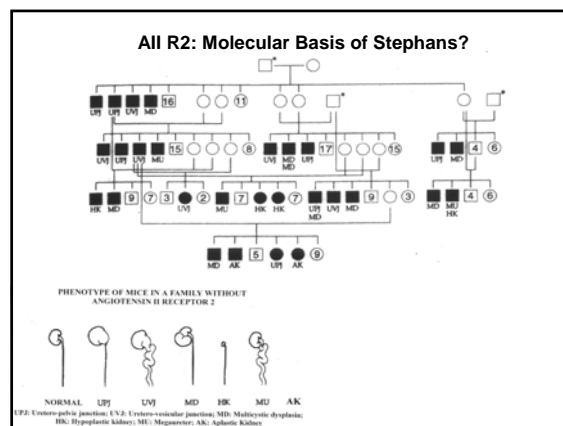
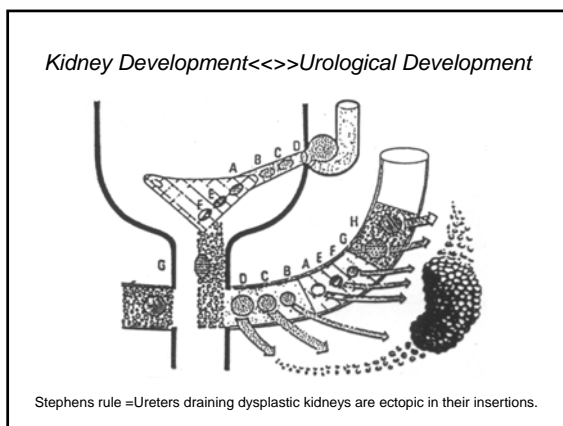
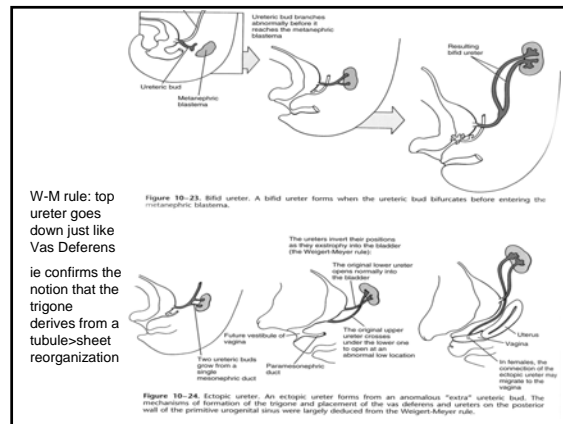
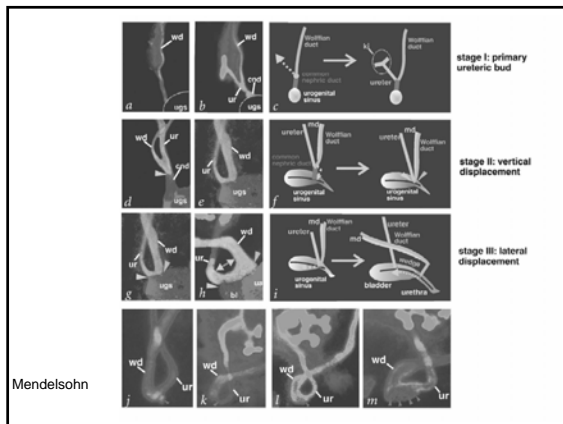
Cortical growth of the 15th branch—produces 10 lateral branches



- ### Two Pathways to Form Epithelia in the Metanephric Kidney
- Mesenchymal Cells into Epithelial Cells-Nephron, A Sequence
 1. Proliferation of mesenchymal cells.
 2. De novo expression of competency factors (Pax-2, WT-1).
 3. De novo expression of conversion factors (Wnt-4).
 4. De novo expression of junctional proteins (NCAM>Cad6>E-cadherin>ZO-1).
 5. De novo expression of matrix proteins (collagen I/III, Fn > laminin alpha5, collagen IV).
 6. De novo expression of cytoskeletal proteins (vimentin>cytokeratin).
 - Branching Morphogenesis- Ureteric Bud, A Sequence
 1. Proliferation of epithelia
 2. Transient loss of epithelial organizers such as E-cadherin
 3. But retention of other components of polarity such as Zo-1
 4. Coherent growth of the tubule.
 5. Distinct populations of tip cells which can act as pathfinders, or have different rates of replication. Ret*
 6. 3 types of branching-terminal bifid, arcade and cortical lateral-Regulatory events?

- ### Concepts
- Lineage marker
 - Epiboly and experiments that investigated the "duct".
 - The plasticity of the duct systems.
 - Mesenchymal to epithelial conversion
 - Induction and Reciprocal Induction
 - Monopodial & dipodial branching
 - Stephen's Hypothesis and the "shared molecule hypothesis"





- Kidney Development <=> Urological Development
- How to make a nephron
 - How to make 10^6 nephrons.
 - How to make the connection from kidney to bladder.
 - How nephron formation is related to hypoplasia and dysplasia and how these are related to abnormalities of the urinary track.

Concepts

- Nephron number is variable, hypoplasia, dysplasia
- Mesenchymal to epithelial conversion
- Lineage marker
- Epiboly and experiments that investigated the "duct".
- The plasticity of the duct systems.
- Induction and Reciprocal Induction
- Monopodial & dipodial branching
- Stephen's Hypothesis and the "shared molecule hypothesis"

Epithelia

- Mesenchymal to Epithelial Conversion
- Tubule elongation and branching
- Tubules-sheets of cells
- Tubules being used for multiple purposes.