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CORRELATIVE EXCRETORY MECHANISMS

The excretory system (urinary system in amniotes) has two major functions: conservation of water and maintenance of chemical equilibrium in the body fluids. In accomplishing these missions, "wastes" are excreted. Frequently, what is "waste" at one moment (for instance, excess salts) may be highly desirable the next. Aquatic animals in a marine environment, freshwater organisms, and terrestrial animals have unique problems with respect to water conservation. The limiting membrane of an organism, the epidermis or skin, gills and lungs, as well as excretory structures all play a role in water conservation and excretion of "wastes."

Color the excretory structures of the invertebrates on the left side of the plate and related titles. After coloring, read below.

Paramecia possess *contractile vacuoles* whose membranes are permeable to water. As water is absorbed through the cell membrane, the appropriate osmotic pressure within the cell may be adversely altered. The *vacuole* takes up the water through the central portion and excretes it to the outside via feeder canals.

In many other invertebrates with relatively small surface areas and volumes, wastes in the form of ammonia or urea, carbon dioxide, and so forth are simply excreted by diffusion pressure through the cell membrane. In roundworms and many insects, urea and other waste products are absorbed by *excretory canals* or *tubules* and delivered to the outside through a pore or are excreted into the intestine for discharge through the anus. In flatworms and proboscis worms a network of ciliated cells or nests of cells (flame cells or bulbs) absorb waste products from the body cavity. These wastes are conducted by ducts to one of several pores along the length of the body. Such a closed tubular system, derived from ectoderm, is called a *protonephridial* system.

Other worms and certain molluscs possess nephridial tubules that are open directly into the coelom or remnants of the coelom (for example, the pericardial cavity). These constitute *metanephridia* and are often located segmentally throughout the length of the organism or are enclosed in a single unit that opens into a bladder (as seen in the clam). The *metanephridium* consists of a convoluted, ciliated tubule open to the coelom at the nephrostome and open to the outside at the nephridiopore. Derived from ectoderm, the *metanephridia* filter waste products from the coelomic fluid and reabsorb necessary water and other chemicals required at the moment.

Color the structures of the vertebrate kidneys and read below.

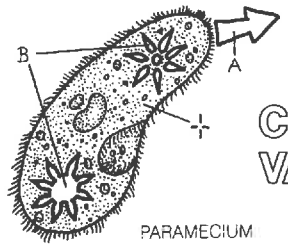
The vertebrate ***kidney*** is formed from mesoderm. The embryonic vertebrate ***kidney*** lies along the length of the dorsal body wall. Its head end (head ***kidney, pronephros***) survives for only a short period during embryonic development and is represented, in part, by convoluted, ciliated tubules that open into the coelom at one end and form an ***archinephric duct*** at the other end. These are not homologous with the ***metanephridia*** of invertebrates. The rest of the ***kidney (opisthonephros)*** consists of ciliated convoluted tubules, arranged segmentally, forming large capsules at one end (which are in contact with blood vessels) and emptying into the ***archinephric duct*** at the other end. Such an ***opisthonephric kidney*** is characteristic of a jawless fish (hagfish).

The ***kidney*** of the shark, bony fishes, and amphibians is located along the dorsal body wall of the abdominal cavity and is somewhat shorter than that seen in the agnates. The pronephros does not exist in the adult but survives long enough embryologically to form the archinephric duct. In the male the ducts of the testes make connection with the nephric ducts in the anterior part of the opisthonephros (not shown). The archinephric duct is thus a urogenital duct. The anterior part of the opisthonephros is often degenerated to one degree or another. The tubules of the main ***kidney*** are compacted together and are no longer arranged segmentally. The adult ***kidney*** (functional opisthonephros) of jawed fishes and amphibians is often called the *mesonephros* and the archinephric duct is called the *mesonephric duct.*

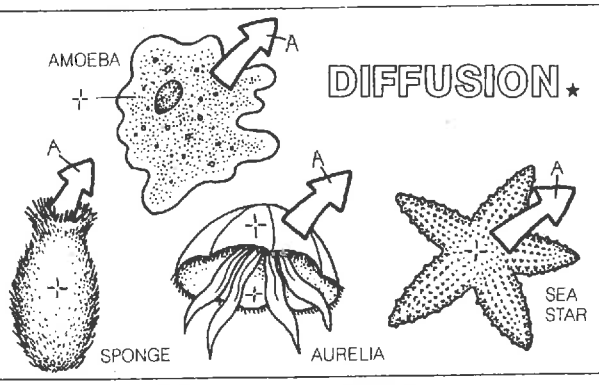
In the mammalian ***kidney*** the posterior part of the opisthonephros (metanephros) persists while the remainder largely degenerates in embryonic development. In males the archinephric duct retains its connection to the testis and becomes the epididymis and ductus deferens. The metanephros becomes a bean-shaped mass lateral to the gonads on the dorsal body wall. It consists of a million or so highly compacted ***nephrons*** connected to ***collecting ducts*** and intimately associated with tufts and networks of blood vessels. Tiny branches of the renal arteries form convoluted tufts (glomeruli) invaginated into hollow capsules of the *nephrons.* The blood plasma is filtered into the capsule, and the filtrate passes through the nephric tubule, where 99 percent of it is reabsorbed by the tubule cells. Only undesired materials of the moment, dissolved in a small amount of water, form urine, which is discharged into the calyces of the ***kidney*** from the collecting ducts. Urine passes through the *ureter* (a specific urinary duct associated only with the metanephros) into the urinary ***bladder*** and out the *urethra.*

CORRELATIVE EXCRETORY/URINARY MECHANISMS.

INVERTEBRATE — EXCRETION$_A$ — VERTEBRATE



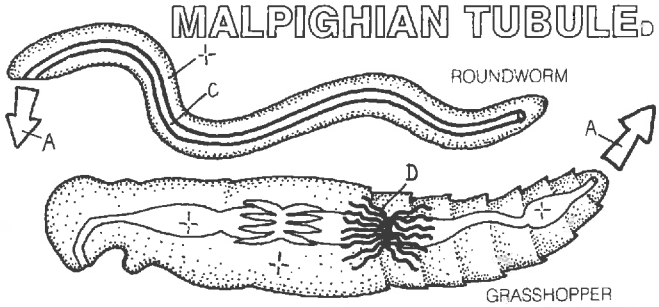
CONTRACTILE VACUOLE$_B$



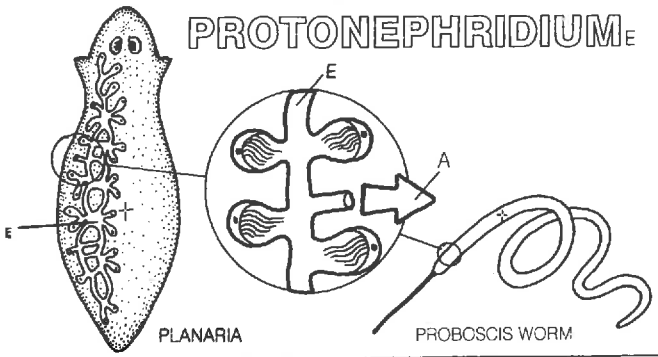
DIFFUSION ★

EXCR. CANAL$_C$

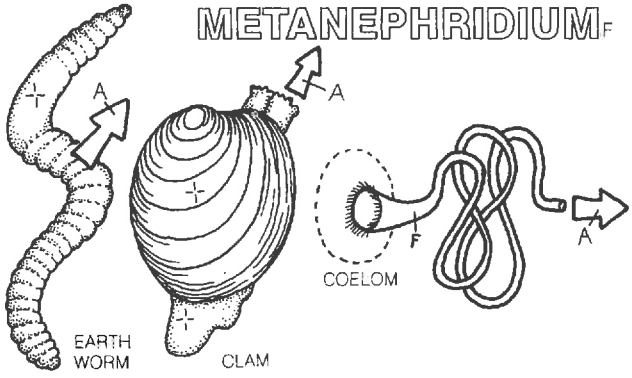
MALPIGHIAN TUBULE$_D$



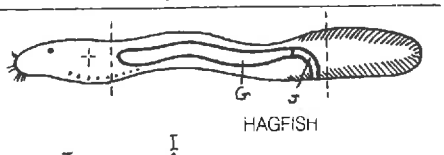
PROTONEPHRIDIUM$_E$

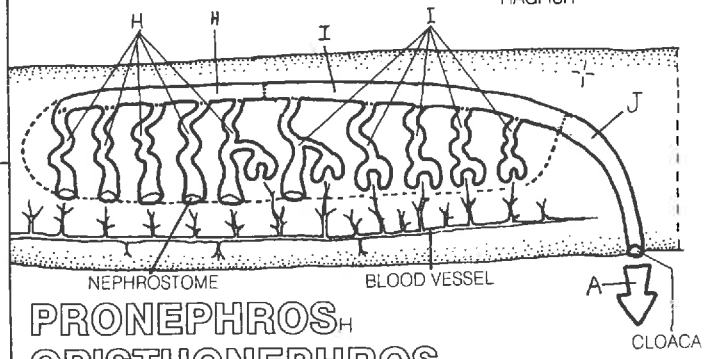


METANEPHRIDIUM$_F$



KIDNEY$_G$



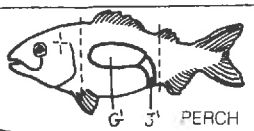


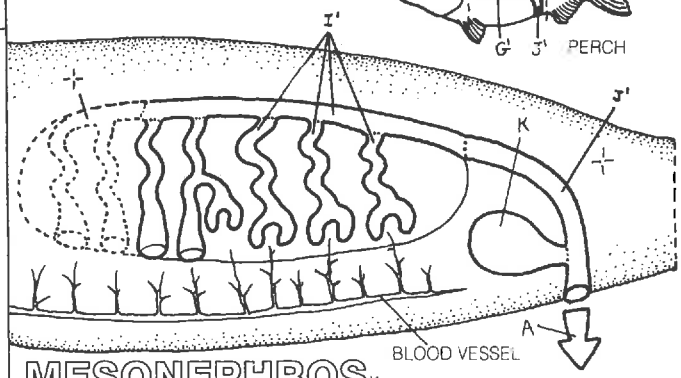
PRONEPHROS$_H$

OPISTHONEPHROS$_I$

ARCHINEPHRIC DUCT$_J$

KIDNEY$_{G'}$





MESONEPHROS$_{I'}$

MESONEPHRIC DUCT$_{J'}$

BLADDER$_K$

URINARY SYSTEM ★

KIDNEY$_{G^2}$

NEPHRON$_L$

COLLECT. DUCT$_M$

URETER$_N$

BLADDER$_{K'}$

URETHRA$_O$

INTRODUCTION TO KIDNEY STRUCTURE

Kidneys produce urine. Under normal resting conditions, the kidneys, which comprise less than 0.5% of the body weight, receive 25% of the cardiac output! Each minute some 1300 ml of blood enters the kidneys through the *renal arteries,* and 1298–1299 ml leaves via *renal veins,* with the difference, 1–2 ml, leaving as urine via the *ureter.* Why all this fuss (claiming one quarter of the body's blood supply) for a measly 2 ml of urine? What does urine contain and why is its formation so important?

At first glance, the composition of the urine is not impressive: water, salt, small amounts of acid, and a variety of waste products, such as urea. What is impressive is how urine composition and volume *change* to compensate for any fluctuation in volume or composition of body fluids. The composition of the body fluids is apparently determined not by what the mouth takes in but by what the kidneys keep. While the design of the gastrointestinal tract appears to maximize absorption, its role in regulation is minimal. The kidneys are the guardians of the internal environment; they rework the body fluids fifteen times a day. When the body is dehydrated, the volume of water excreted decreases; when body fluids become more acid, kidneys excrete more acid; if the K^+ content of body fluids rises, the kidneys excrete more K^+. "We have the kind of internal environment we have because we have the kidneys we have" —Homer Smith.

THE WHOLE KIDNEY

The kidneys are about the size of a clenched fist. They lie against the back abdominal wall, just above the waistline. The outer covering of the kidney, called the *capsule,* is thin but tough and fibrous. When it is cut open, two regions appear: an outer zone (the *cortex)* and an inner region (the *medulla).* A microscopic view reveals the unit of kidney function, the *nephron.* Each kidney has about 1 million nephrons, which are tubular structures about 45 to 65 mm long and about .05 mm wide. Their walls are made of a single layer of epithelial cells.

NEPHRONS

A funnel-like structure about 0.2 mm in diameter, called *Bowman's capsule,* comprises the top end of the nephron. These capsules are always found in the cortex. Fluid flows through the lumen of the tubule from Bowman's capsule into the next section, the *proximal tubule,* which has a "curly" or convoluted portion and then a straight segment that dips into the medulla. This section, about 15 mm long, is called the proximal tubule because it is near the origin of the nephron (Bowman's capsule). Fluid then flows into a long, thin tube that plummets straight toward the depths of the medulla. This is the descending limb of the *loop of Henle.* At its lowest point, the loop makes a hairpin turn and begins to ascend out of the medulla back toward the cortex, becoming considerably thicker toward the latter portions of its ascent. In the cortex, the ascending limb of the loop becomes continuous with the distal tubule. Finally, the distal tubule empties into the *collecting duct,* a tube that gathers fluid from several nephrons.

There are two major classes of nephrons. The majority, called cortical nephrons, originate in the outer portions of the cortex and are characterized by short loops of Henle that reach only the outer regions of the medulla. The remaining nephrons, which comprise only about 15% of the total, originate closer to the medulla and are known as juxtamedullary nephrons. These have very long loops of Henle that reach deep into the medulla; they are important for water conservation in the body.

COLLECTING DUCTS

Individual collecting ducts coalesce into larger tubular structures, and this pattern repeats until several of the larger tubes empty into a still larger funnel structure, the *renal pelvis.* Fluid in the renal pelvis is identical to urine. The renal pelvis is continuous with the ureter, which leaves each kidney to convey urine to the bladder, where it is stored until elimination via the urethra.

BLOOD SUPPLY TO NEPHRONS: 2 CAPILLARY BEDS IN SERIES

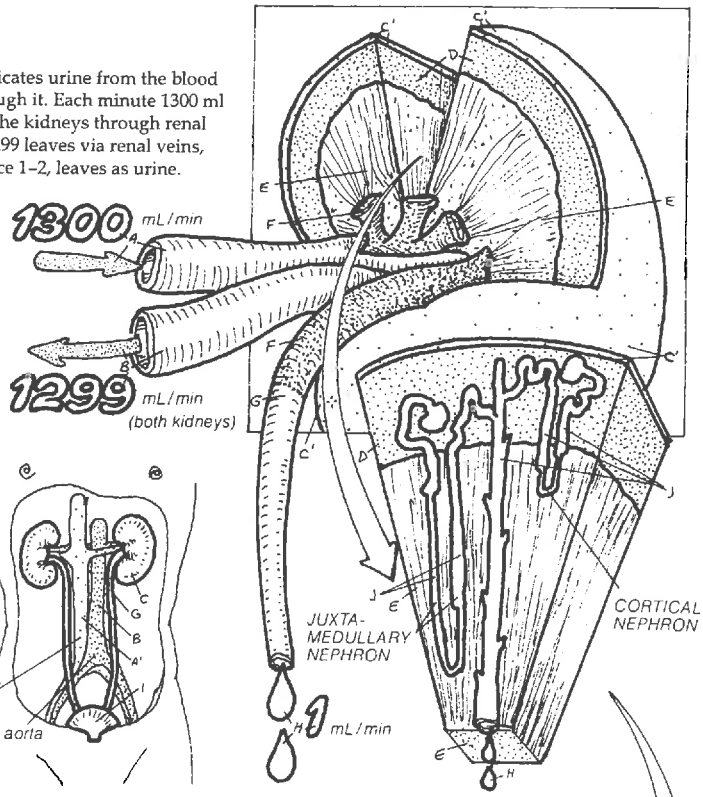
The blood supply to the nephrons is special because it consists of two capillary beds in series. Each Bowman's capsule has its own capillary bed, called a *glomerulus.* (Sometimes the combined structure, Bowman's capsule + glomerulus, is referred to as the glomerulus.) The vessel bringing blood to the glomerulus is called the *afferent arteriole.* Blood leaving the glomerulus does not enter a venule; rather, it enters another arteriole, the *efferent arteriole,* which serves as a conduit to the second capillary bed, called *peritubular capillaries.* The peritubular capillaries are so interconnected that it is difficult to tell which capillary came from which efferent arteriole; the tubules of any one nephron probably receive blood from several efferent arterioles. Efferent arterioles from juxtamedullary nephrons also form peritubular capillaries in much the same way, but they also send off branches—straight tubes that follow descending limbs of Henle's loops deep into the medulla, turn at the bend of the loop, and ascend back toward the cortex. These hairpin loops of blood vessels are called *vasa recta;* their design is important for water conservation.

By the time the fluid in the nephron has passed through the collecting ducts to reach the pelvis, it has become urine. Plate 59 shows how fluid simply filters out of the glomerular capillaries into Bowman's capsule. From here, it flows along the lumen of the nephron and is modified by the epithelial cells of the tubules and the collecting ducts until it finally becomes urine.

CN: Use red for A structures, blue for B, purple for R, and yellow for H. Use dark colors for J and T.
1. Begin with the cut-away drawing of the kidney in the upper right. Color the section of a kidney showing the location of two types of nephrons. Note that these have been greatly enlarged for diagrammatic purposes.
2. Color the enlarged view of a kidney section in the lower right corner. Begin with the entry of blood (A^1) at the bottom and color the arteries and arterioles. Note that the afferent and efferent arterioles have been given different colors (P & Q) to distinguish them from the other vessels. Color the blood circulation before coloring the structures of the nephron—K–O.
3. Color the lower left diagram of the glomerulus and the flow of filtrate through the nephron. Color in the Bowman's capsule (K) first.

RENAL ARTERY A
RENAL VEIN B
KIDNEY C
CAPSULE C
CORTEX D
MEDULLA E
RENAL PELVIS F
URETER G
URINE H
BLADDER I
NEPHRON J
BOWMAN'S CAPSULE K
PROXIMAL TUBULE L
LOOP OF HENLE M
DISTAL TUBULE N
COLLECTING DUCT O

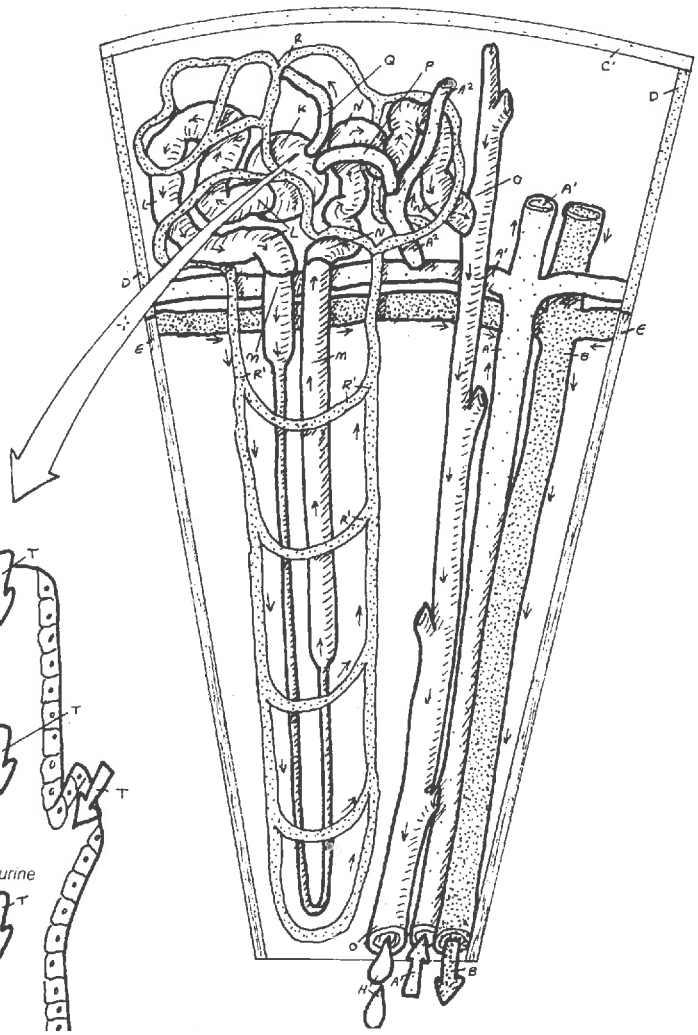
The kidney fabricates urine from the blood that passes through it. Each minute 1300 ml of blood enters the kidneys through renal arteries, 1298–1299 leaves via renal veins, and the difference 1–2, leaves as urine.



Each kidney has about 1 million tubular nephrons, functional units that produce urine from a filtrate of blood. Each nephron contains a filtering part—Bowman's capsule—followed by a long tubular part consisting of the proximal tubule, loop of Henle, distal tubule, and collecting duct. Fluid in Bowman's capsule is protein-free plasma. Fluid at the end of the collecting duct is urine.

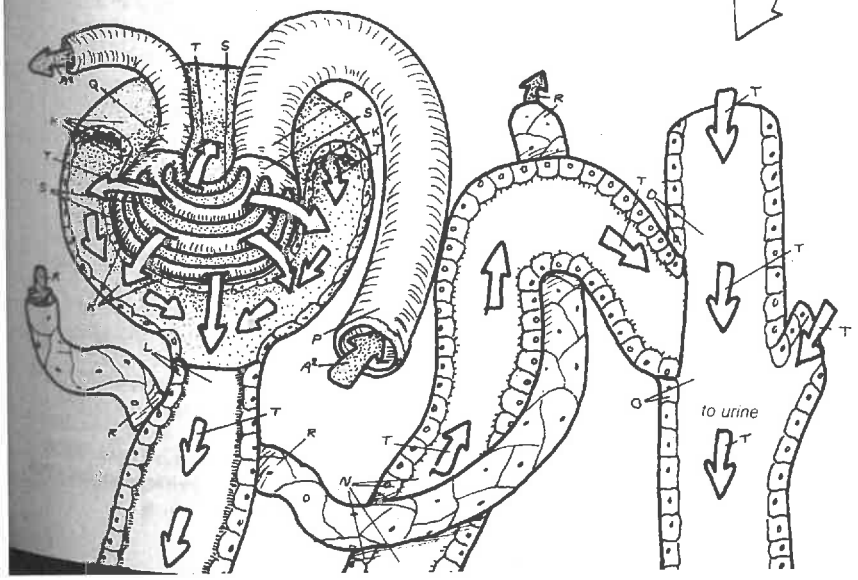
ARTERY A'
ARTERIOLE A²
AFFERENT ARTERIOLE P
EFFERENT ARTERIOLE Q
PERITUBULAR CAPILLARY R
VASA RECTA R'

Blood supply to the nephrons (seen on the right) consists of two capillary beds in series. The afferent arteriole conveys blood to the glomerulus (seen below) lying in Bowman's capsule. Blood then flows through the efferent arteriole and empties into the peritubular capillaries to supply the proximal and distal tubules in the cortex. The medulla is supplied by branches of the efferent arteriole from juxtamedullary nephrons. These branches, the vasa recta, plunge into the medulla, and following the loop of Henle, make hairpin turns and return to the cortex.



GLOMERULUS S
FILTRATE T
BOWMAN'S CAPSULE K

Fluid filters through the glomerulus into Bowman's capsule of the nephron. The filtrate then continues through the tubules on its way to the collecting duct. During this process, nutrients and most of the fluid are withdrawn; the composition of the remaining fluid is further modified until it becomes urine at the end of the collecting duct.



FILTRATION, REABSORPTI AND SECRETI

By the time fluid in the nephron passes through the collecting ducts to reach the pelvis, it has become urine. What is this fluid in the nephron and how did it get there? The unusual pattern of blood circulation to the kidney provides a clue. Rarely do we find one capillary bed (the *glomerulus*) leading into an arteriole (*efferent arteriole*), which in turn leads into another capillary bed (*peritubular capillaries*). The pressure in a typical capillary located elsewhere in the body begins around 35 mm Hg and falls some 20 mm until it reaches 15 mm Hg at the venous end of the vessel. If glomerular capillaries were typical, pressure of the blood delivered to the efferent arteriole would be only 15 mm Hg, hardly enough to drive blood through the next vessel, the narrow efferent arteriole. Thus, the pressures in these capillary beds must be atypical.

BLOOD PRESSURE: HIGH IN GLOMERULUS, LOW IN PERITUBULAR CAPILLARIES

Pertinent data for normal kidney blood pressures in humans are unavailable, and it is difficult to obtain accurate values even in animals, where measurements are compromised by anesthesia, surgical trauma, and blood loss. The best figures for monkeys, dogs, and rats indicate that glomerular blood pressure is high, around 50 mm Hg. The pressure drop in passing through these capillaries is small, only a few mm Hg; apparently, glomerular capillaries have a low resistance. But like that of any arteriole, the resistance of the efferent arteriole is considerable; by the time blood reaches the peritubular capillaries, the pressure has fallen to about 15 mm Hg.

Glomerular filtration: ultra filtrate of blood plasma enters the nephron—What physiological significance can we attach to these aberrant pressures? Glomerular pressures are abnormally high; peritubular pressures are abnormally low. Fluid transfer across capillary walls is determined by the balance of capillary *blood pressure* and *oncotic pressure,* so the high glomerular pressure suggests that a net fluid *filtration* occurs at these capillaries and that fluid within Bowman's capsule is simply a *filtrate* of blood plasma (i.e., fluid that would be obtained from blood if it were strained through a porous filter, in this case the porous walls of the glomerular capillary). This has been verified experimentally. Fluid at the beginning of the nephron does not arise out of any active transport process; proteins and cells are simply separated from the plasma by a passive filtration process.

High pressure and high permeability ensure glomerular filtration—This filtration is the first step in modifying a portion of blood plasma that will eventually be excreted as urine. As the fluid flows along the nephron past the cells making up the tubular walls, substances may be withdrawn from the fluid and returned to the blood via the peritubular capillaries; this process is called *reabsorption.* Alternatively, some substances may be removed from the blood and added to the tubular fluids in a process called *secretion.* Glomerular filtration followed by tubular reabsorption and secretion are the fundamental processes by which the kidney regulates the internal environment.

The funnel-like structure of *Bowman's capsule* allows it to collect the filtrate and convey it to the lumen of the proxima tubule. Fluid that filters from the blood into the lumen of th nephron must pass through three potential barriers: (1) the thin cell layer making up the capillary wall (the capillary endothelium), (2) the *basement membrane* associated with the capillary, and (3) the epithelial cell layer making up Bowman's capsule. The capillary endothelium is riddled with *fen estrations,* which are easily penetrated by most molecules bu not by cells. The basement membrane and the outer surfaces of both cell layers are embedded with glycoproteins that con tain a strong negative charge. The last barrier, those epitheli cells of Bowman's capsule that are in direct contact with the capillaries, have a peculiar structure; they are called *podocytes* Podocytes send out foot processes that interdigitate with foo processes of other cells. Vacant spaces or *slits* remain betwee these foot processes. The filtration pathway through the capillary fenestrations, across the basement membrane, and through the slit passages does not hinder the passage of smal molecules like salts, glucose, and amino acids. As the size of the molecules increases, the pathway begins to offer some resistance, depending on the molecule's size, shape, and electrical charge. Electrically neutral molecules, the size of the plasma protein albumin, can permeate this barrier to a limitec extent. However, albumin, which is negatively charged, is restrained by the negative charge on the basement membrane and cell surfaces.

The glomerular capillaries not only have a higher pressure, they are also more permeable than many capillaries. Both factors promote filtration. Glomerular capillaries filter twenty times more fluid than ordinary capillaries. Fully one-fifth of the fluid entering the capillary is delivered to the nephron via the filtration path. This loss of fluid from the blood concentrates the remaining proteins. By the time blood enters the efferent arteriole and peritubular capillaries, the oncotic pressure (osmotic pressure exerted by the plasma proteins) has risen from a normal value of 25 to 30 mm Hg.

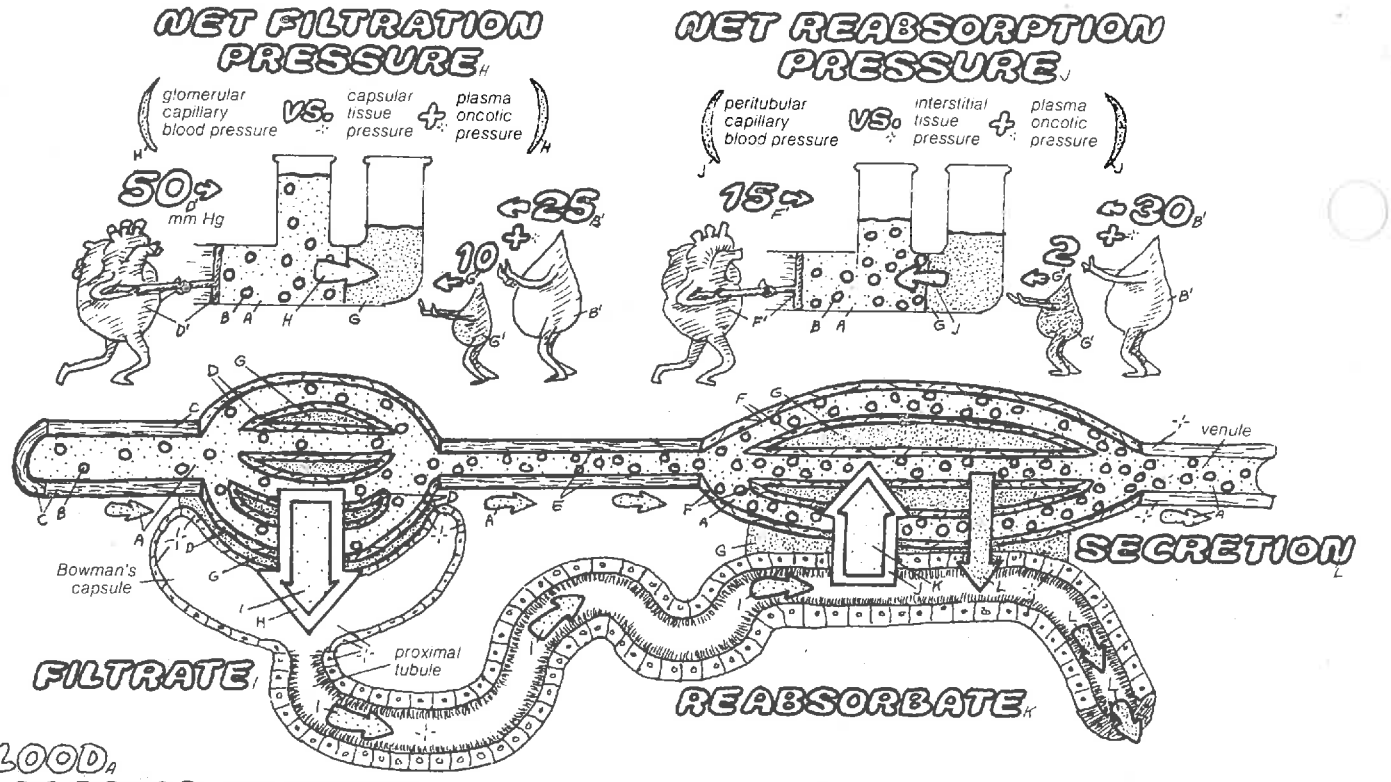
LOW PRESSURE FAVORS PERITUBULAR REABSORPTION

Just as high pressure prepares the glomerulus for filtration, low pressure in the peritubular capillary promotes fluid reabsorption over its entire length. The major force for reabsorption from interstitial spaces arises from the osmotic pressure of the plasma proteins, and we have seen that this is unusually high in peritubular capillaries. Because the opposing filtration pressure (blood pressure in the capillary) is low, the peritubular capillary is well adapted to reabsorb fluid from its environment. However, these arguments apply only to reabsorption between capillary and interstitial fluids. Fluid reabsorption between nephron and interstitial space is governed by a more complex set of osmotic forces determined by the concentrations of small solutes, especially NaCl. Compared to capillaries, nephron walls are much less permeable to small solutes, so the contribution of these small solutes to effective osmotic pressure gradients becomes large.

CN: Use red for A, light blue for G, and dark colors for H, J, and L.

1. Begin with the diagram of filtration, reabsorption, and secretion by coloring the horizontal blood vessels, blood, and plasma proteins before coloring the upper diagrams.

2. Color the elements of the renal corpuscle, be ning with the small exterior diagram, then the functional diagram on the far left, and then the interior view. Notice that the slit pore (P), repr sented by arrows, refer to a narrow space betw the cells of the podocytes (P).

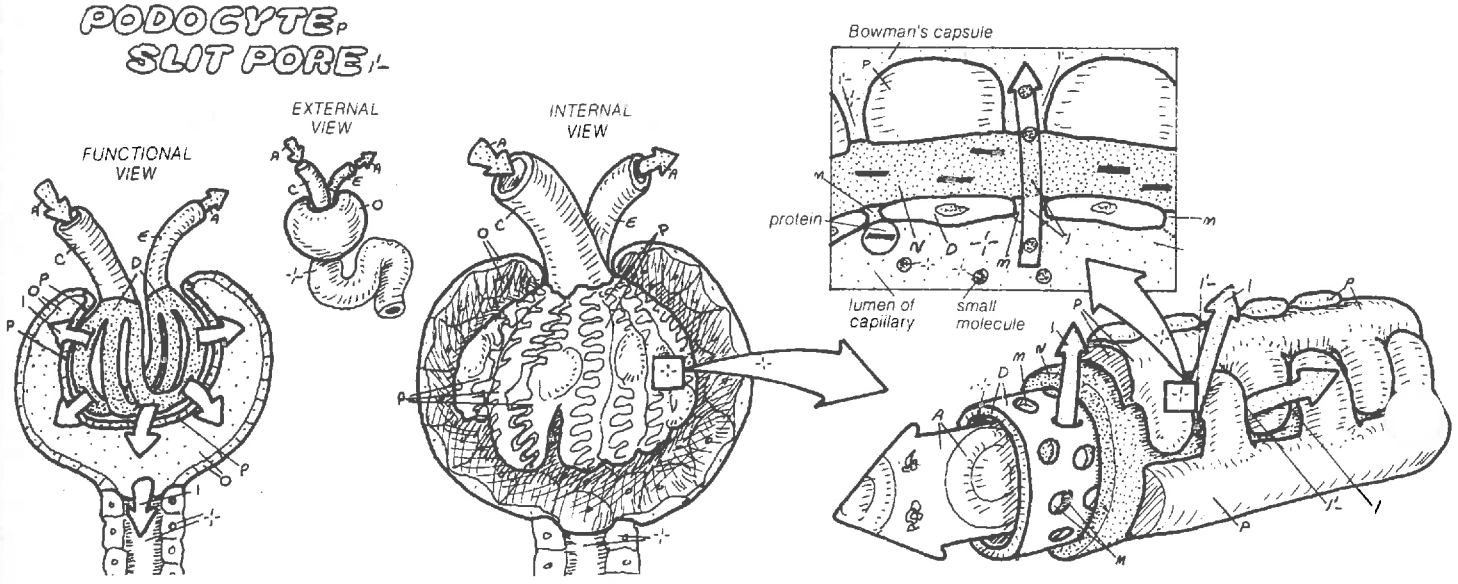


BLOOD A
PLASMA PROTEIN B
AFFERENT ARTERIOLE C
GLOMERULAR CAPILLARIES D
PRESSURE I
EFFERENT ARTERIOLE E
PERITUBULAR CAPILLARIES F
PRESSURE F'
INTERSTITIAL FLUID G
PRESSURE G'
ONCOTIC (OSMOTIC) GRADIENT B'

Net filtration occurs at the glomerular capillaries, where blood pressure is abnormally high, while net reabsorption occurs at the peritubular capillaries, where pressure is low. Fluid filtering out of the glomerular capillaries concentrates the plasma proteins and raises the average oncotic pressure of peritubular capillaries a few mm Hg. In a typical glomerular capillary, blood pressure = 50, tissue pressure = 10, and oncotic pressure averages 25 mm Hg. Net filtration pressure = [50 – (10 + 25)] = 15 mm Hg. In a typical peritubular capillary, blood pressure = 15, tissue pressure is small, possibly 2, and oncotic pressure averages 30 mm Hg. Net reabsorption pressure = [(2 + 30) – 15] = 17 mm Hg. Fluid obtained from Bowman's capsule is identical to blood, with no cells and no protein. As fluid flows in the nephron, substances may be withdrawn and returned to the blood via peritubular capillaries, a process called reabsorption. Other substances may be removed from blood and added to tubular fluids, a process called secretion.

RENAL CORPUSCLE
GLOMERULUS O
CAPILLARY FENESTRATIONS n
BASEMENT MEMBRANE N
BOWMAN'S CAPSULE O
PODOCYTE P
SLIT PORE P'

To filter from the blood to a nephron, a substance must pass through two cell layers plus the basement membrane that separates them. Blood cells and proteins are too large to pass, but smaller substances can. Capillary walls have many fenestrations and are very permeable. The basement membrane is also very permeable; it contains fixed negative charges that help repel plasma proteins (also negatively charged). The last cell layer, called podocytes, has slit-like pores between cells. Podocytes are part of Bowman's capsule.



FUNCTIONS OF THE PROXIMAL TUBULE

Approximately 120 ml of protein-free plasma filters into the nephrons each minute. If this fluid were excreted as urine, it would take only 25 minutes (3000 ml plasma/120) to exhaust the entire plasma volume. This fluid would carry with it everything dissolved in the plasma (glucose, amino acids, minerals, vitamins, etc.). The fact that you are reading this page is living proof that this does not happen. The tubules recapture (reabsorb) most of the fluid, practically all the nutrients, and some minerals before the filtrate reaches the ends of the collecting ducts.

The nephron is primarily a regulatory organ. Faced with a torrent of fluid at its origin, its first job is to reduce the volume of filtrate to manageable levels and to reclaim essential nutrients. The responsibility for this task falls primarily on the proximal tubule. By the time the filtrate reaches the end of the proximal tubule, two-thirds of the water and virtually all of the nutrients have been reabsorbed. Of the original 120 ml of fluid that entered through the filter, only 40 ml passes on to the loop and distal tubule where more subtle regulatory processes take place.

Tubular Cells Are Asymmetric

This massive transport requires asymmetric tubular cells. Note in the bottom diagram that the cell membrane facing the lumen is covered with fingerlike projections called *microvilli*. They resemble bristles in a brush; hence the membrane is called the brush border. The membrane surrounding the remaining three-quarters of the cell has no microvilli; it is called the *baso-lateral* membrane (plate 2). These two membranes have different properties—they contain different proteins, enzymes, and transport systems. The two membranes are separated by *tight junctions* that prevent migration of any proteins from one membrane to the next. The baso-lateral membrane resembles membranes of most cells—e.g., it contains many *Na^+-K^+ pumps* and *facilitated diffusion* systems for glucose and amino acids (plates 9, 10). The brush border does not contain these transporters, but it contains others.

Active Na^+ Transport Drives Water Reabsorption

The prime mover for most proximal tubular transport is the active transport of Na^+ (via the Na^+-K^+ pump), which keeps intracellular Na^+ concentration more than ten times lower than extracellular. Because Na^+ concentration is higher in the lumen than in tubular cells, it moves down its concentration gradient via several different co- and counter-transporters into the cell (see below). But it cannot be pumped back out into the lumen because the brush border has no Na^+ pump; it can be pumped out of the cell into the interstitial spaces only by the baso-lateral membrane. The result is a stream of Na^+ moving from lumen to cell, only to be pumped out into the interstitial space, where it can diffuse into the peritubular capillary. In other words, Na^+ is reabsorbed. But Na^+ carries a positive charge; it attracts negatively charged ions that, one way or another, move along with it. Because Cl^- is the most abundant, easily transported negative ion, we end up reabsorbing large quantities of Na^+ and Cl^-.

Both Na^+ and Cl^- are important determinants of the effective osmotic gradients across the tubular cell. Each time a Na^+ and Cl^- are transported from lumen to interstitial space, the lumen loses two osmotically active particles, while the interstitial space gains two. This creates an osmotic gradient favoring reabsorption of water. For each Na^+ and Cl^- moved, about 370 water molecules follow to maintain osmotic equilibrium. Once the water arrives in the interstitial space, the high oncotic pressure (and low blood pressure) in the peritubular capillaries is sufficient to absorb the water back into the blood. The loss of water from the tubular lumen concentrates the remaining solutes, and those that are freely permeable to tubular membranes will diffuse down the resulting concentration gradient from lumen to interstitial space. So in addition to reabsorption of Na^+, the asymmetric Na^+ transport is also responsible for reabsorption of Cl^-, copious amounts of water, and some fraction of other diffusible solutes.

Secondary Active Transport of Glucose, Amino Acids, Lactate, and Phosphate

In addition to Cl^- and water, Na^+ transport is also coupled to the reabsorption of glucose, amino acids, lactate, and phosphate. The brush border contains a different system to *co-transport Na^+* with each of these substances (plate 9). Since these systems operate similarly, we shall describe only Na^+ and glucose. This system transports Na^+ and glucose together, but will not operate with either alone. The system is symmetric; it does not require ATP, and it is capable of transporting the pair into or out of the cell. In practice, the co-transport system always transports the pair into the cell because of the Na^+-K^+ pump, which keeps intracellular Na^+ scarce and makes it difficult for glucose to find an Na^+ partner to ride the co-transport system in the reverse direction. By keeping intracellular Na^+ low, the cell creates a one-way system for glucose transport. As a result, glucose accumulates inside the cell even above its concentration in the lumen or plasma; it is as though glucose has been actively transported into the cell. And it has, in a way, only now the energy has come from the Na^+ gradient and only indirectly from the splitting of ATP. It is an example of secondary active transport.

Once glucose is inside the cell in higher concentrations, it moves out through the baso-lateral membrane toward the blood via a facilitated transport system that does not require Na^+. The transport of amino acids, lactate, and phosphate are similar.

Other Functions of the Proximal Tubule

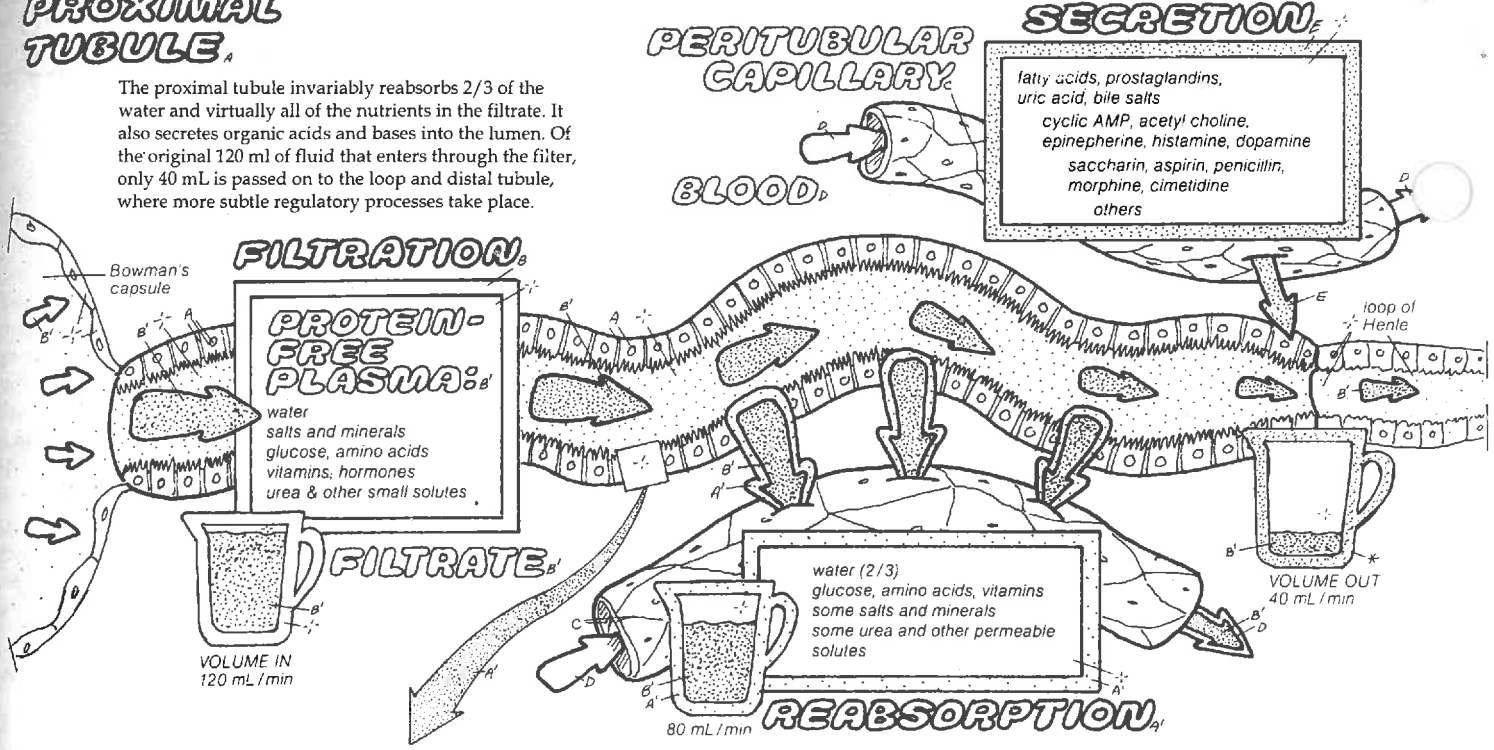
Proximal tubules also play a role in acid-base balance (plate 64) and in regulating calcium, magnesium, and phosphorus. In addition, they have active transport systems for *secretion* of organic acids and bases from blood to lumen. This system is important clinically because many drugs afect it. The secretory transporter is often located on the baso-lateral membrane, so the secreted material is accumulated in the cell. The brush border passively transports these substances; they move from the cell, where they are concentrated, to the lumen.

CN: Use the same colors as on the previous plate for proximal tubule (A), filtration (B), secretion (E), and capillary (C).

1. Begin with the upper diagram, coloring the filtrate (B^1) entering the proximal tubule (A) on the far left.
2. In the lower diagram, first color the filtrate arrow, the tubular cells (A^2) with their brush borders (F), and the capillary wall on the right. The follow the order of the titles and color the variou transport mechanisms. Note that the substances being transported receive the color of that partic lar mode of transport, so that it is possible for N to receive four different colors. Start with the AT driven sodium pump at the asterisk in the lowe right corner of the center cell.

PROXIMAL TUBULE A

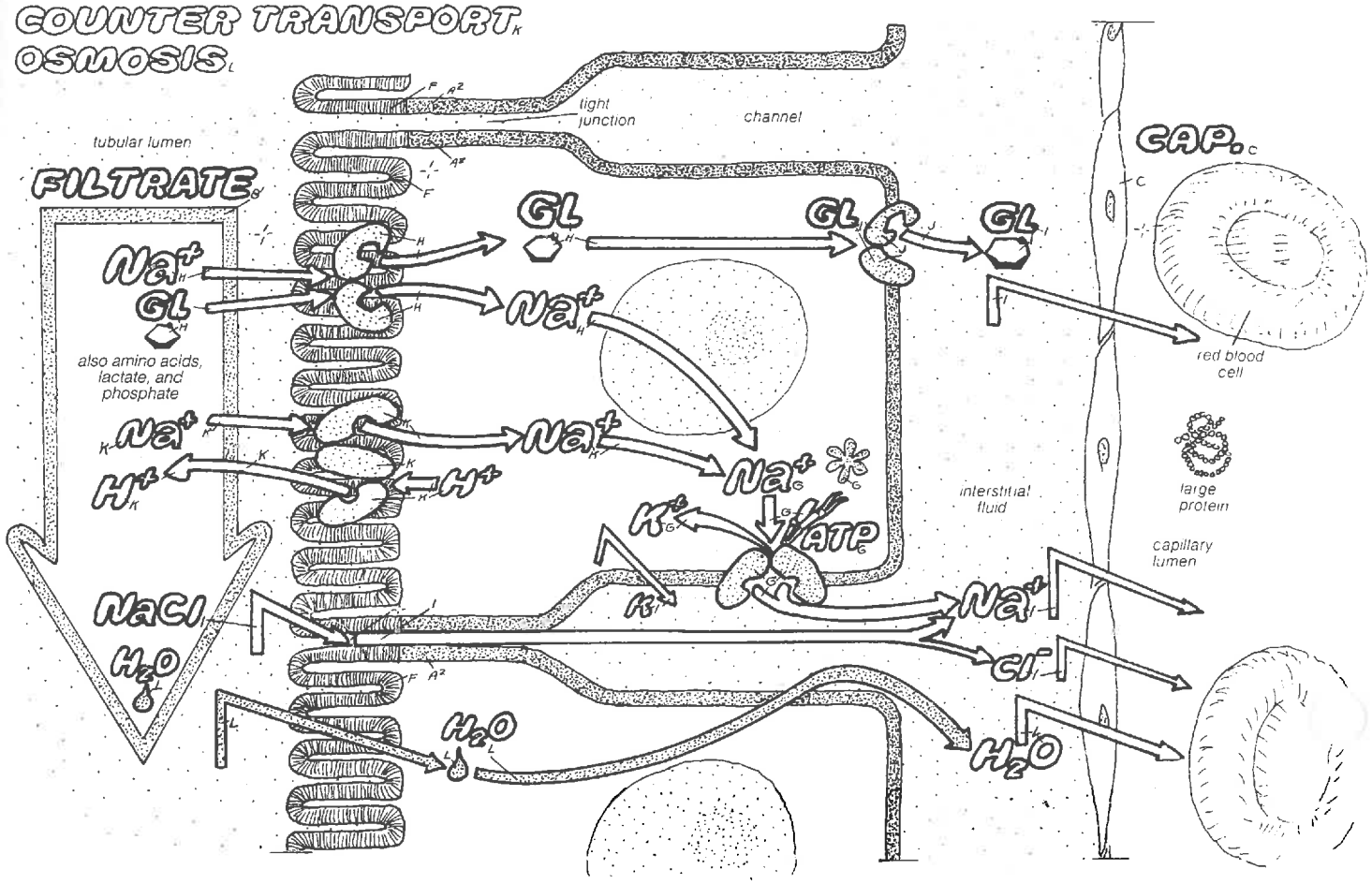
The proximal tubule invariably reabsorbs 2/3 of the water and virtually all of the nutrients in the filtrate. It also secretes organic acids and bases into the lumen. Of the original 120 ml of fluid that enters through the filter, only 40 mL is passed on to the loop and distal tubule, where more subtle regulatory processes take place.



TUBULAR CELL A'

BASOLATERAL MEMBRANE A"
BRUSH BORDER (MICROVILLI) F
ACTIVE TRANSPORT G
CO-TRANSPORT H
DIFFUSION I
FACILITATED DIFFUSION J
COUNTER TRANSPORT K
OSMOSIS L

Most reabsorptive processes are coupled to movements of Na^+. Na^+ is pumped out of the tubular cell by an Na^+-K^+ pump located in the basolateral membrane, but not in the brush border. This provides a one-way movement from lumen to blood. Cl^- follows Na^+ because of electrical attraction. Movement of Na^+ and Cl^- creates an osmotic gradient that drags water with it. By linking with downhill Na^+ movement into the cell, other solutes are pumped uphill. Glucose and amino acids are co-transported with Na^+ into the cell. H^+ is countertransported out of the cell (in exchange for Na^+).



MEASURING FILTRATION, REABSORPTION & SECRETION

In modern times it has been possible to micro-dissect the kidney in anesthetized animals, collect fluid at different positions in individual nephrons, and tease out portions of nephrons to study them in detail. However, techniques for the study of quantitative aspects of the whole kidney have been available for many years. The latter techniques are particularly valuable because they are non-invasive and can be readily applied to unanesthetized humans.

BOOKKEEPING IN THE KIDNEY

The principle used to measure filtration, reabsorption, or secretion is simple: what goes in must come out. It is an application of the conservation of matter. Suppose you knew that 100 mg of a sugar were filtering into the nephrons each minute, but only 60 mg were appearing in the urine. Unless the nephrons were destroying the sugar, 40 mg (100 - 60) were reabsorbed. Alternatively, if 120 mg appeared in the urine, you would conclude that 20 mg (100 - 120 = -20) had been secreted during that minute.

Amount excreted per minute = $U_s \times V$—How do we estimate how much goes through the filter and how much comes out in the urine during each minute? The latter is easy. Collect the urine over a period of time—say an hour. Analyze it to find out how much sugar there is in each milliliter (i.e., determine the concentration of the sugar in the urine), then multiply this figure by the total number of milliliters of urine collected. This gives the amount excreted per hour. To find the amount excreted per minute, divide by 60. Letting E = the amount of a solute excreted per minute, U_s = the concentration of the solute in the urine, and V = the volume of urine that is excreted per minute, we have

$$(1) \quad E = U_s \times V.$$

Amount filtered per minute = P_s x GFR—Estimating the amount of solute going through the filter each minute (called *filtered load*) is trickier. A related quantity, the number of milliliters of fluid flowing through the filter each minute, is called the glomerular filtration rate, abbreviated as GFR. If we knew the GFR, the problem would be easier. Let P_s = the concentration of any solute, such as sugar, in the blood plasma. Then the amount of sugar coming through the filter each minute (i.e., the filtered load), F, will be given by

$$(2) \quad F = P_s \times GFR.$$

For our final bookkeeping on tubular activities (reabsorption or secretion), we let RS_s denote the amount that is reabsorbed (or secreted) during each minute. Then

$$(3) \quad RS_s = F - E = [P \times GFR] - [U_s \times V].$$

If RS_s is positive, it represents reabsorption. If it is negative, it represents secretion.

Inulin clearance measures the GFR—Using equation (3), we can calculate RS_s, provided we can measure all the quantities on the right-hand side. Three of these—P_s, U_s, and V—are routine. The fourth, GFR, is not. Turning the problem inside out, if we knew RS_s for any substance, we could solve for GFR. Fortunately, these substances exist; inulin is one of them.

Inulin is a nontoxic polysaccharide that is small enough to pass freely through the filter but too large to pass through solute channels in cell membranes or through the tight junctions between tubular epithelial cells. Further, inulin is not lipid soluble, so it won't permeate the lipid bilayer portion of the cell membrane. Finally, inulin is not produced or metabolized in the body; there are no special transport systems that will carry it. In particular, the tubules neither secrete nor reabsorb inulin; $RS_{inulin} = 0$. Using this fact, we rewrite equation (3) for the special case of inulin: $0 = [P_{in} \times GFR] - [U_{in} \times V]$. Solving for GFR:

$$(4) \quad GFR = [U_{in} \times V] / P_{in}.$$

In practice, GFR is measured by injecting inulin, collecting and analyzing blood and urine samples at intervals, and using this last expression for calculation. For historical reasons, the ratio $[U_s \times V] / P_s$ for any substance s is called the *clearance* of S. The GFR is equal to the *inulin clearance*. Notice that GFR is simply the amount of fluid flowing through the filter per minute. It really has nothing to do with inulin. Inulin is merely an artificial substance that we use to trace the filtrate so we can measure its volume. To study a more interesting solute—call it S—we follow the same routine, only now we analyze the blood and urine for both inulin and S. Inulin data are used to calculate GFR from equation (3) as before, and this result, together with the blood and urine data for S, is used in equation (3) to calculate RS_s. These procedures have been used both clinically to test renal function and experimentally to study renal mechanisms.

Renal tubular cells "renew" the plasma every 25 min—Through the use of inulin clearance, an estimate of a normal value for GFR = 120 ml/min (both kidneys) has been obtained. This means that each day 120 × 60 × 24 = 172,800 ml of fluid passes through the glomerular filter into the lumens of the nephrons, a space that is essentially outside the body. That is an enormous amount of fluid, a volume approximately three times the total volume of all the body fluids. It means that the entire plasma volume (approximately 3000 ml) passes through the nephrons every 25 min (3000 /120)! That is, by selective reabsorption and secretion, the renal tubular cells renew the plasma every 25 min.

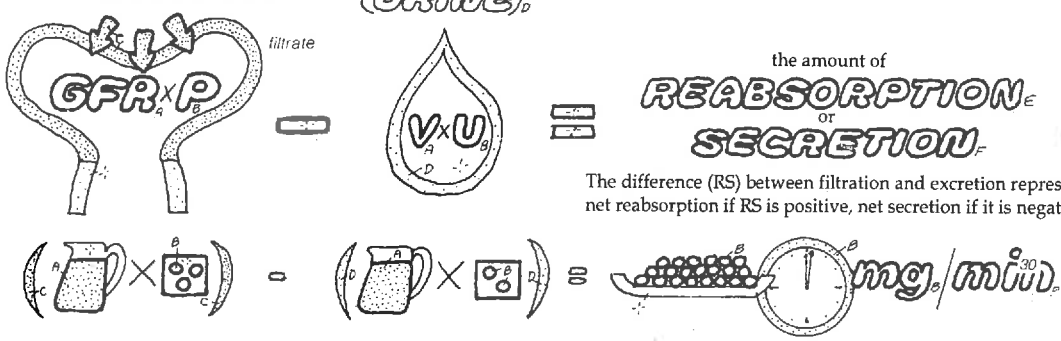
Glucose reabsorption is limited—A typical example of the use of clearance techniques is provided by the glucose excretion experiment illustrated in the lower diagram. Various amounts of glucose were administered to systematically change plasma glucose concentrations from normal to very high. At normal levels (70–110 mg/100 ml) and below, no glucose is excreted; the entire filtered load is reabsorbed. As plasma concentration is increased, so is the filtered load. Eventually, we reach a threshold plasma concentration where almost all reabsorption sites are working to maximal capacity, and some glucose escapes reabsorption, spilling over into the urine. The maximal capacity to reabsorb glucose is called the *TM (tubular max)*. The diagram shows how reabsorption for glucose (RS_{GL}) changes with plasma concentration. It is obtained by subtracting E from F at each concentration.

CN: Use the same colors as on the previous page for filtration (C), reabsorption (E), and excretion (D). Use light blue for A and a dark color for B. Note that the two shapes at the top are Bowman's capsule (filtration) and a drop of urine (excretion).
1. Begin with the formula at the top.
2. Color the three central panels, starting on the left. Note that in the first two panels the number boxes filtering is kept artifically small for purpos of simplicity.
3. Color the procedure for the measurement of glucose reabsorption into the blood stream.

WATER (mL)$_A$
SOLUTE CONCENTRATION (mg/mL)$_B$

Kidney performance can be assessed by simple bookkeeping—i.e., measuring the net balance between inflow through the filter (filtration) and outflow into the urine (excretion). For each substance (S), inflow = number of ml of plasma filtering in per min (GFR) × amount of S contained in each ml. Outflow = number of ml of urine excreted per min × amount of S contained in each ml of urine.

FILTRATION$_C$ EXCRETION$_D$ (URINE)$_D$

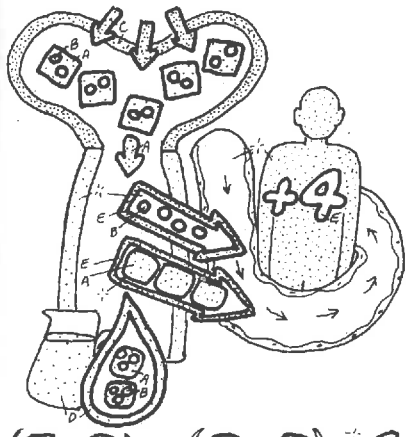


the amount of

REABSORPTION$_E$ or SECRETION$_F$

The difference (RS) between filtration and excretion represents net reabsorption if RS is positive, net secretion if it is negative.

FILTRATION & REABSORPTION$_E$

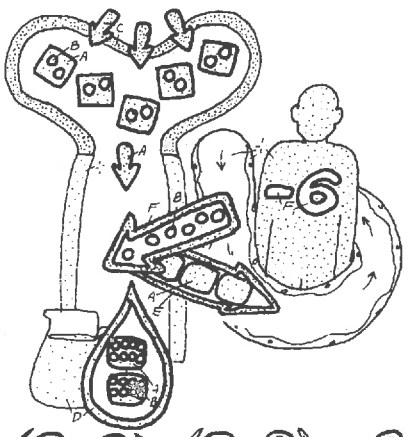


$(5_A \times 2_B)_C - (2_A \times 3_B)_D = 4_E$

REABSORPTION$_E$

Count the number of solute particles (circles) filtering in. Each box of water contains two, and there are five boxes per min (GFP), so that filtered load = 5 × 2 = 10 per min. Similarly, there are 2 × 3 = 6 particles leaving (excreted) per min. The difference, four particles, is reabsorbed (RS = 10 – 6 = 4 particles per min).

FILTRATION & SECRETION$_F$



$(5_A \times 2_B)_C - (2_A \times 8_B)_D = -6_E$

SECRETION$_F$

Count the number of particles filtering in: 5 × 2 = 10 per min. Similarly there are 2 × 8 = 16 particles leaving per min. The balance (difference) is negative (RS = – 6). Six particles are secreted per min.

FILTRATION ALONE$_C$

$(120_A \times 2_B)_C - (1_A \times 240_{B'})_D =$

REABSORPTION = 0$_E$

SECRETION = 0$_F$

Inulin is a special substance; it is not reabsorbed and not secreted (RS_{in} = 0), and filtered load = excretion. We use this fact to measure GFR. Inulin is injected into plasma, and samples of plasma and urine are taken for analysis. If 240 inulin particles are excreted per min and each ml of plasma contains only two, then we require 120 ml of plasma per min to deliver the 240 particles being excreted. GFR = 120 ml per min. The algebra is shown below.

$$(GFR_A \times P_{B'})_C - (V_A \times U_{B'})_D = 0_E$$

$$GFR_A = \frac{V_A \times U_{B'}}{P_B}$$

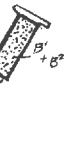
$$GFR_A = C_{IN_{B'}}$$

(CLEARANCE OF INULIN$_{B'}$)

MEASURING GLUCOSE$_{B^2}$ REABSORPTION$_E$ (RS_{GL})$_E$

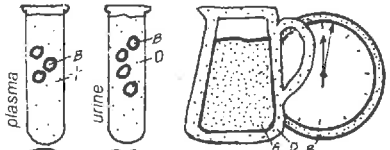
To study how glucose reabsorption depends on plasma glucose:

1$_{B^2}$ INJECT: GLUCOSE$_B$ + INULIN$_{B'}$



2$_{B^2}$ GFR$_A$ = $\frac{V_A U_{IN_{B'}}}{P_{IN}}$

2$_{B^2}$ TAKE SAMPLES & MEASURE



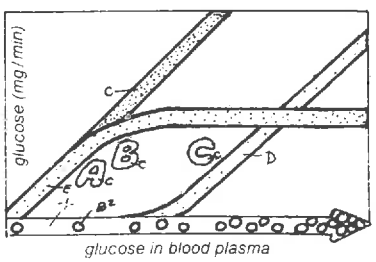
P_{GL_B} U_{GL_B} V_A

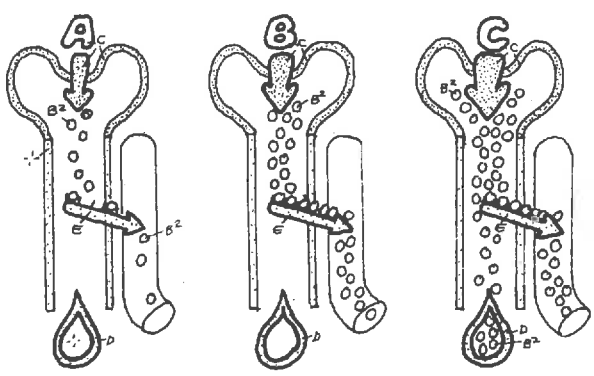
P_{IN_B} U_{IN_B}

Calculate the GFR from measured values of P_{in}, U_{in}, and V. Use this value of GFR together with measured values of P_{gl}, U_{gl}, and V to calculate RS_{gl}.

4$_B$ $(GFR_A \times P_{GL})_C - (V_A \times U_{GL})_D = RS_{GL_E}$

A plot of results of these measurements at different plasma glucose concentrations (P_{gl}) is shown here. RS_{gl} is obtained by subtracting the amount excreted from the amount filtered at each concentration. At normal levels (A) and below, no glucose is excreted; the entire F is reabsorbed. Increasing P_{gl} increases F and RS_{gl}. Eventually a P_{gl} is reached where almost all reabsorption sites are working to maximal capacity (B) and some glucose spills over into the urine (C). Increasing F beyond this level cannot increase RS_{gl}.





REGULATION OF THE GFR

Control of the *glomerular filtration rate (GFR)* is crucial to kidney performance. Abnormally fast filtration will swamp the tubules, allowing filtrate to speed by the cells before they have time to modify the fluid. Abnormally slow rates will compromise the kidneys' ability to process adequate amounts of fluid during each minute. Nevertheless, blood pressure and blood flow to the kidney do change, often in response to stresses not directly related to kidney function (e.g., a sudden drop in arterial pressure; see plate 45). How can changes in GFR be modulated under these circumstances?

SYMPATHETIC NERVE IMPULSES CONSTRICT RENAL ARTERIOLES

Panel A shows that renal blood flow is reduced by, *sympathetic nerve* impulses, which constrict arterioles, but the effect of these impulses on GFR depends on which arterioles are most constricted. Constricting the *afferent arteriole* reduces renal blood flow, causing downstream (glomerular) pressure to decrease, thereby decreasing GFR. Constricting the *efferent arteriole* also reduces renal blood flow, but now the glomerulus is upstream. Its pressure rises and GFR increases. Because the afferent arterioles contain more smooth muscle, their constriction is generally the more forceful. However, the simultaneous constriction of efferent arterioles can be expected to diminish changes in GFR that might otherwise occur.

RENAL BLOOD FLOW AND GFR ARE INSENSITIVE TO ARTERIAL PRESSURE

Myogenic mechanism—Panel B illustrates an important property of renal blood vessels: both renal blood flow and GFR are very insensitive to changes in systemic arterial blood pressure in the range of 80 to 180 mm Hg. (Compare the flat part of the curves with the dotted diagonal line that would be expected if the blood vessels were simple passive structures.) Shared by most vascular beds, this behavior is most pronounced in the kidneys. Due to properties of the smooth musculature of the vessel walls, this behavior persists when all nerve supplies are cut but disappears when the smooth muscle is paralyzed with drugs. Apparently, the blood vessel smooth muscles are sensitive to pressure. Stretching the muscle opens special stretch-sensitive ion channels that depolarize the cells and the muscle contracts. When pressure rises, flow would ordinarily increase, but contraction of the smooth muscle in the arteriolar wall, reduces the radius of the vessel and increases its resistance. As a result, flow does not increase as much, and energy (pressure) is lost flowing through the high resistance. Thus, capillary pressure and the ensuing GFR do not increase as much.

MATCHING GFR TO REABSORPTION CAPACITY OF INDIVIDUAL NEPHRONS

The kidneys' capacity to regulate body fluids is especially sensitive to the rate at which fluid is delivered to the *distal tubule.* This is where regulation of salt, water, and acidity occurs. If the flow is too fast, the distal tubule cells will be overwhelmed; if it is too slow, there is danger of overcompensation. The lower diagram on the left shows a *feedback* mechanism that adjusts the GFR *in each single nephron* to maintain a constant load delivery to the distal tubule.

The beginning of the distal tubule of each nephron is located next to its corresponding glomerulus and makes contact with the afferent arteriole in a specialized structure called the *juxtaglomerular (JG) apparatus.* As flow increases, solute delivery (probably NaCl) to the JG apparatus increases and in some unknown way stimulates constriction of the afferent arteriole so that GFR in the same nephron decreases. Conversely, as flow decreases, GFR increases. In this way, the GFR is matched to the reabsorption capacity of the proximal tubule. The mechanism, called *tubuloglomerular feedback,* is particularly interesting because, unlike the two mechanisms described above, it is a discrete, local regulation; each nephron has its own independent control system. If, for example, the glomerulus of a particular nephron becomes damaged and leaky so that the filtration rate in that nephron increases, the feedback will constrict the afferent arteriole of that nephron and no others.

MATCHING PROXIMAL FLUID REABSORPTION TO GFR: PHYSICAL MECHANISMS

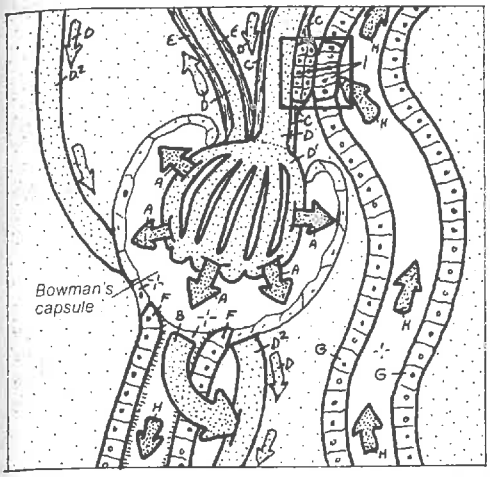
Increased GFR is accompanied by increased peritubular oncotic pressure—There are two simple physical mechanisms that operate to match proximal fluid reabsorption to the GFR. If, for some reason, GFR increases, so does *proximal tubular reabsorption.* If GFR goes down, reabsorption decreases. To understand the first mechanism, illustrated on the bottom of the plate, recall that fluid reabsorption is determined by net Na^+ reabsorption. But net Na^+ reabsorption is given by the difference between active pumping of Na^+ (lumen to interstitial space) and the back-leak of Na^+ through tight junctions (TJ) in the reverse direction. If GFR decreases, compensatory reductions in proximal fluid reabsorption occur because of the following. With a small GFR, less fluid is removed from glomerular capillaries, so the plasma proteins become less concentrated as they flow through the glomerulus. This means that the oncotic pressure delivered to the peritubular capillaries is lowered, reducing the forces favoring fluid reabsorption by these capillaries from the interstitial fluid. The buildup of fluid in the interstitial space will increase the tissue pressure, which may force the seal between cells (the tight junction) to leak so that both water and Na^+ leak back into the tubular lumen. The steps are reversed when GFR increases, resulting in a compensatory increase in reabsorption.

Distant portions of the proximal tubule provide a reserve for reabsorption under increased loads—The second mechanism that helps match changes in tubular reabsorption to changes in GFR depends on the coupling of fluid reabsorption to solute reabsorption, particularly to Na^+, which is co-transported with glucose, amino acids, and other solutes. With normal GFR, these co-transported nutrients are completely reabsorbed before they reach the end of the proximal tubule. With higher GFR, more solute is filtered and the more distant reaches of the tubule begin to be utilized. More solute is reabsorbed so more fluid is also reabsorbed. Those distant portions of the proximal tubule not used to transport glucose or amino acids during normal GFR supply a reserve for reabsorption under increased loads.

CN: Use red for the D letters including RBF (renal blood flow—D^3). Use dark colors for A and B.
1. After noting the question posed at the top of the page, color the titles 1 (above) and 2 (below) titles 1 (above) and 2 (below), which are the two major categories covered. Begin coloring in the upper left corner, which defines the area of the nephron that deals with the subject matter of this page. Note the area enclosed in the rectangle in the upper part of the illustration. It represents the cells of the juxtaglomerular apparatus (I), which is composed of part of the distal tubule (G) and the afferent arteriole (C).
2. Color the systemic sympathetic (J) control of the GFR.
3. Color example B, noting the sharp rise in both RBF and GFR when the arterial blood pressure exceeds 180 mm Hg.
4. Color the response of distal tubule feedback to a rise in the GFR. Begin with the GFR.
5. Color the bottom panel, completing the left example first.

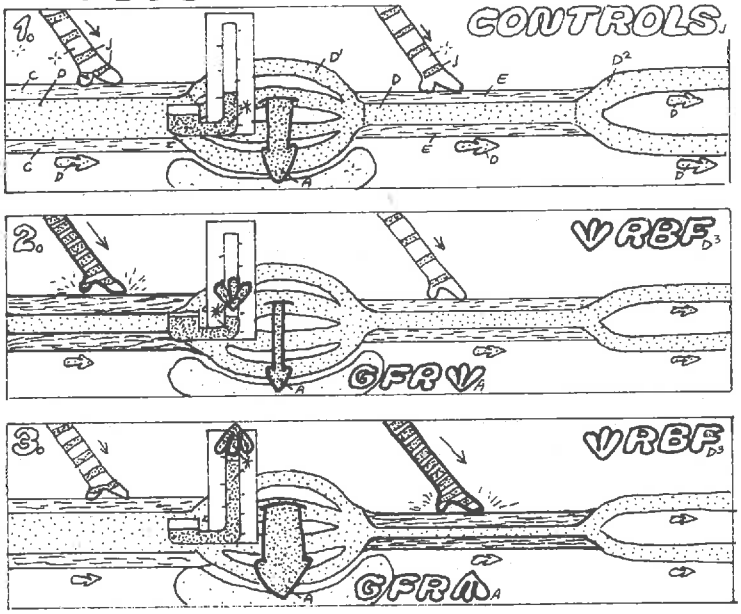
HOW IS FLOW TO THE DISTAL TUBULE REGULATED?

1 REGULATE GLOMERULAR FILTRATION RATE (GFR)$_A$



AFFERENT ARTERIOLE$_C$
BLOOD$_{A,B}$ GLOMERULUS$_D$
EFFERENT ARTERIOLE$_E$
PERITUBULAR CAPILLARY$_B$
PROXIMAL TUBULE$_F$
DISTAL TUBULE$_G$
SOLUTES$_H$
JUXTAGLOMERULAR APPARATUS (JG)$_I$

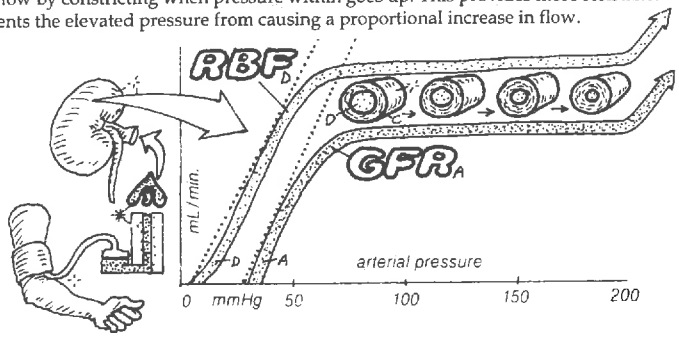
A SYSTEMIC SYMPATHETIC CONTROLS$_J$



Renal blood flow (RBF) is controlled by sympathetic nerve impulses that constrict arterioles, but their effect on GFR depends on which arterioles are constricted the most. Constricting the afferent arteriole reduces RBF, causing downstream (glomerular) pressure to decrease, thereby decreasing GFR (2). Constricting the efferent arteriole also reduces RBF, but now the glomerulus is upstream. Its pressure rises and GFR increases (3).

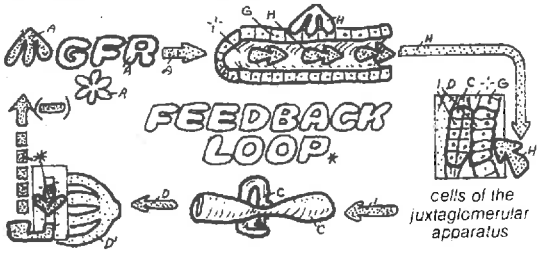
B LOCAL RESPONSE TO ARTERIAL PRESSURE CHANGE

RBF and GFR are insensitive to changes in systemic arterial pressure (compare with dotted line that would be expected if blood vessels were simple passive structures). Blood vessels regulate flow by constricting when pressure within goes up. This provides more resistance and prevents the elevated pressure from causing a proportional increase in flow.



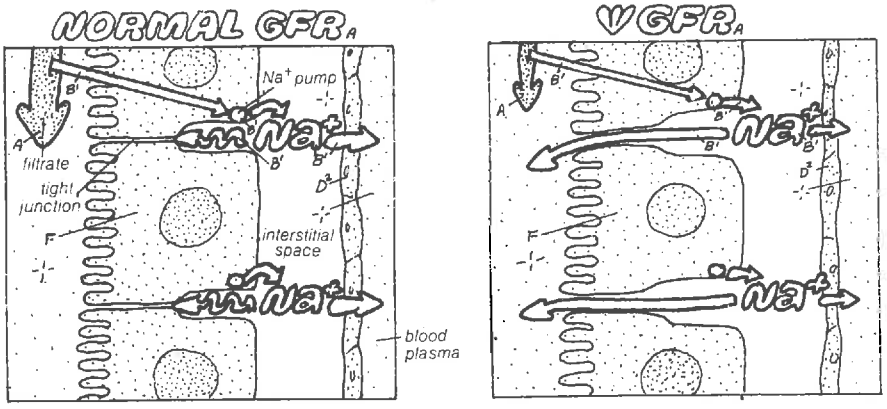
C TUBULOGLOMERULAR FEEDBACK$_G$

GFR in each single nephron is adjusted to maintain a constant delivery to the distal tubule by negative feedback. As flow increases, solute delivery to the JG apparatus increases and stimulates constriction of the afferent arteriole, so that GFR in the same nephron decreases.



2 REGULATE PROXIMAL TUBULE REABSORPTION$_B$

Fluid reabsorption is determined by net Na^+ reabsorption, which is the balance between active pumping of Na^+ (lumen to interstitial fluid, ISF) and back-leak of Na^+ through tight junction (TJ) in the reverse direction. If GFR decreases, compensatory decreases in proximal fluid reabsorption occur because less filtration → lower concentration of plasma proteins (oncotic pressure) delivered to peritubular capillaries → less capillary reabsorption of fluid from ISF → increased pressure in ISF → increased back-leak of Na^+ through TJ → less net Na^+ reabsorption → less fluid reabsorption. Conversely, an increase in GFR induces a compensatory increase in reabsorption.



WATER CONSERVATION & ANTIDIURETIC HORMONE

Animals living in fresh water are continuously challenged with water balance problems. Their plasma has a high solute concentration *(osmolarity)* and tends to draw water by osmosis from its surroundings. They cope with a continuous inundation of water by excreting large volumes of it. Animals living on land—including humans—have the opposite problem. Their environment is arid, and they face the threat of drying up. To conserve water, birds and mammals excrete very small volumes of concentrated urine, but how?

LOOP OF HENLE CREATES A HYPERTONIC INTERSTITIAL SPACE Only birds and mammals excrete urine that is *hypertonic* (more concentrated than their plasma). Only birds and mammals have long loops of Henle. Further, those species with more highly developed loops are capable of excreting more concentrated urine. These observations led earlier investigators to suggest that the formation of hypertonic urine takes place in the loops of Henle. This idea was shattered by the first micropuncture studies of the distal tubule, which contains the fluid just after it has passed through the loop of Henle. This fluid is always *hypotonic* or at most isotonic, but never hypertonic, as required by the hypothesis. Apparently, hypertonic urine must be formed in the collecting duct. The loops of Henle are involved in a more subtle way. By actively pumping NaCl without allowing water to follow, the loops of Henle create a unique *hypertonic interstitial fluid* in the deep portions of the *medulla.* Collecting ducts pass through this fluid on the way to the ureter and take advantage of their hypertonic surroundings by allowing water to be withdrawn by osmosis from the lumen of the duct to the interstitial space.

Ascending limb of loop of Henle actively reabsorbs Na^+, leaves water behind to deliver hypotonic fluid to the distal tubule—The loops of Henle of juxtamedullary nephrons plunge into the depths of the medulla. These descending limbs are fairly permeable to Na^+ and water and do not appear to have any special properties. Once around the bend in the loop, however, the tubules become *water impermeable,* a property that extends well into the distal tubule. Further, the ascending limb actively transports NaCl from the lumen into the interstitial fluid. The major portion of this transport takes place in the thick (upper) portions of the ascending limb, which has cells richly endowed with mitochondria—i.e., ATP producers. (Na^+ crosses the luminal membrane via a co-transport system that moves Cl^- and K^+ as well as Na^+ into the cell. Once inside, Na^+ is pumped out by the $Na^+ - K^+$ pump in the basolateral membrane. Cl^- follows through a passive channel, preserving electroneutrality; the result is transport of NaCl.)

Final adjustment of urine volume and concentration occurs in the collecting ducts—Although the ascending limbs transport NaCl, their membranes prevent the usual concomitant transport of water, so fluid delivered to the distal tubule is hypotonic regardless of the final composition of the urine. This transport of NaCl (out of the water-impermeable ascending limb) without water creates a unique hypertonic interstitial fluid in the medulla. Collecting ducts from *all* nephrons pass through these fluids on their way to the ureter. If the hormone *ADH (antidiuretic hormone, vasopressin)* is present, the latter portions of the distal tubule and the entire collecting duct become water permeable. As fluid flows through these sections of the *distal nephron* (distal tubule and collecting ducts), water equilibrates with the surrounding interstitial fluids. Therefore, as fluid descends via the collecting ducts into the medulla, it becomes more and more concentrated (hypertonic) until urine leaving the collecting duct has the same hypertonic osmolarity as the interstitial fluid in the lower medulla. In fact, the osmolarity of the medulla sets the limit to which urine can be concentrated. ADH also makes the last portions of the collecting duct permeable to urea, which becomes trapped in the interstitial fluid and makes a substantial contribution to its osmolarity.

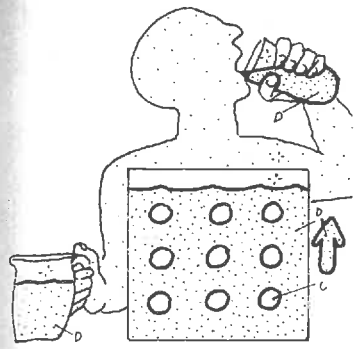
When ADH is absent, as occurs in the disease *diabetes insipidus,* the distal tubule and collecting duct are practically impermeable to water. The hypotonic fluid delivered to the distal tubule becomes even more hypotonic as salts are reabsorbed (without water being able to follow). Fluid reaching the end of the collecting duct is hypotonic, resulting in a large volume of dilute urine. In the disease, lack of ADH causes the kidneys to produce as much as 30 liters of urine per day. One would have to drink more than 120 glasses of water per day simply to keep from drying up.

ADH CONSERVES THE OSMOTIC PRESSURE OF BODY FLUIDS In times of water deprivation, the kidneys conserve water; with the aid of high ADH levels they excrete a low-volume, concentrated (hypertonic) urine. With water intoxication, ADH levels are minimal and the kidneys release the excess water by excreting a high-volume, dilute (hypotonic) urine.

ADH is produced in neural cells of the hypothalamus and is carried via axonal transport to axon terminals in the posterior pituitary, where it is stored. It is secreted from the terminals at the "right time" because neural osmoreceptors, also in the hypothalamus, are very sensitive to the osmotic pressure in the plasma. They respond to small increases in osmotic pressure by increasing their frequency of stimulating impulses sent to the ADH-producing cells. These impulses are relayed down to the axon terminals in the posterior pituitary where they activate channels, allowing Ca^{++} to enter, and stimulate secretion (by exocytosis—plate 20) of the hormone. Thus, a rise in osmotic pressure of the extracellular fluids (reflected in the blood plasma) stimulates cells of the hypothalamus to increase ADH production and to release ADH from the posterior pituitary. ADH travels via the bloodstream to the kidney, where it acts (via cAMP/protein kinase) to insert water channels into the membranes of the distal tubule and collecting duct. This promotes water retention and relieves the rise in osmotic pressure. Hypothalamic osmoreceptors also send excitatory signals to thirst centers in the hypothalamus that initiate sensations of thirst. Drinking more water dilutes the concentration of body fluids. Conversely, when plasma osmotic pressure decreases, secretion of ADH and sensations of thirst diminish.

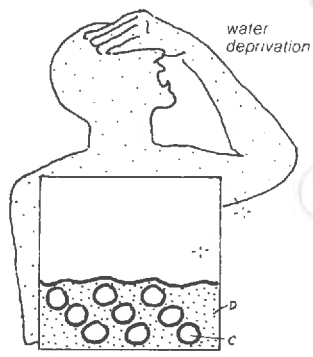
Regulation of body-fluid osmotic pressure is important. When it is low, water is drawn into cells that swell—posing a danger to brain cells, which are confined by the rigid walls of the cranium. Symptoms may include nausea, malaise, confusion, lethargy, seizures, and coma. When osmotic pressure is high, cells shrink; neurological symptoms include lethargy, weakness, seizures, and coma. Sometimes it can even be fatal.

CN: Use light blue for D, a dark color for G, yellow for H, red for I, and purple for J.
1. Begin by coloring the rectangular borders (A and B) of the two large diagrams representing a kidney nephron. They demonstrate which part of the nephron lies within the cortex (A) and which part is in the medulla (B).
2. Color the state of low osmolarity on the left by beginning with the cartoon figure above and then coloring the borders of the nephron itself (D^1 and E). Color all circles and arrows.
3. Do the same for the diagram on the right, noting the ADH influence (G^1) on the collecting duct and a portion of the distal tubule (making the membranes water permeable).
4. Starting with step one, color the lower right illustration, which shows how a rise in osmolarity results in ADH secretion and water reabsorption. Do the summary diagram to the left. The numbers refer to the illustration on the right.

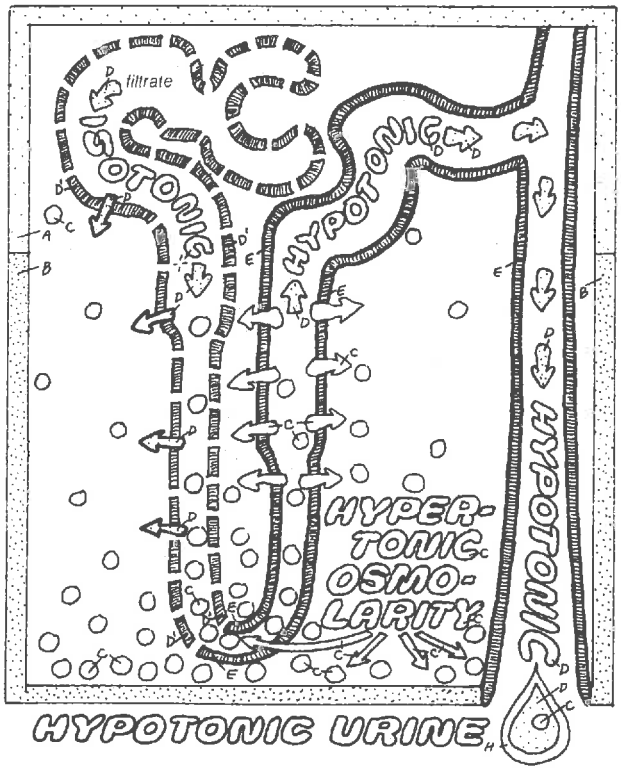


LOW OSMOLARITY$_C$
ADH$_G$

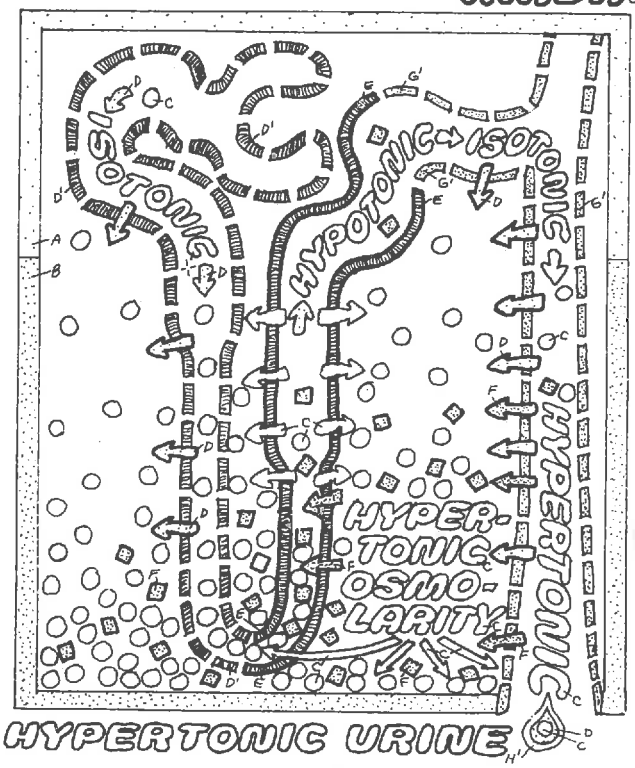
In times of water deprivation (right diagram), the kidneys conserve water; they excrete a low-volume, concentrated (hypertonic) urine. With water intoxication (left diagram), they release the excess water by excreting a high-volume, dilute (hypotonic) urine. To accomplish this task the loops of Henle of juxtamedullary nephrons plunge into the depths of the medulla. The ascending limbs actively reabsorb NaCl, but prevent the usual concomitant reabsorption of water (due to impermeable membranes) so that fluid delivered to the distal tubule is hypotonic regardless of the final composition of the urine. Further, this transport of NaCl (without water following) creates a unique hypertonic interstitial fluid in the medulla. Collecting ducts pass through these fluids on their way to the ureter. If the hormone ADH is present (right diagram), the latter portions of the distal tubule and the entire collecting duct become water permeable. Water equilibrates with interstitial fluid in these sections of the nephron; fluid leaving the distal tubule is isotonic, while fluid leaving the collecting duct has the same hypertonic osmolarity as the interstitial fluid in the lower medulla. When ADH is absent (left diagram), the distal tubule and collecting duct are practically impermeable to water. The hypotonic fluid delivered to the distal tubule becomes even more hypotonic as salts are reabsorbed (without water being able to follow). Urine reaching the end of the collecting duct is hypotonic. ADH also makes the last portions of the collecting duct permeable to urea, which becomes trapped in the interstitial fluid and makes a substantial contribution to its osmolarity.



HIGH OSMOLARITY$_C$
ADH$_G$

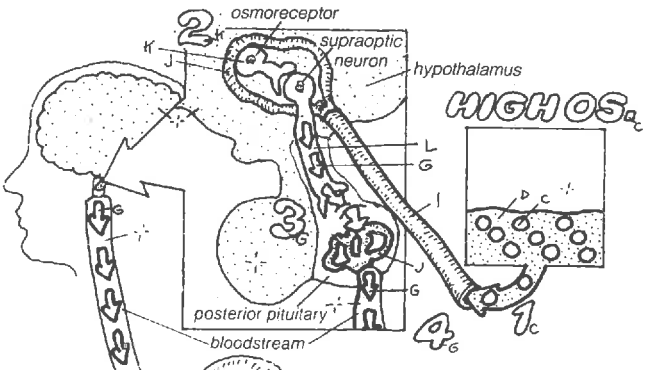


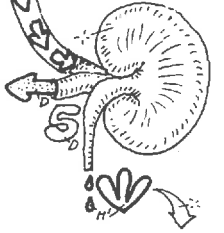
HYPOTONIC URINE$_H$



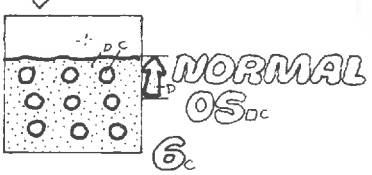
HYPERTONIC URINE$_{H'}$

KIDNEY CORTEX$_A$
KIDNEY MEDULLA$_B$
SODIUM CHLORIDE (NaCl)$_C$
WATER$_D$
WATER PERMEABLE MEMBRANE$_{D'}$
IMPERMEABLE MEMBRANE
UREA$_F$
ANTIDIURETIC HORMONE (ADH)$_G$
INFLUENCE ON MEMBRANE$_{G'}$

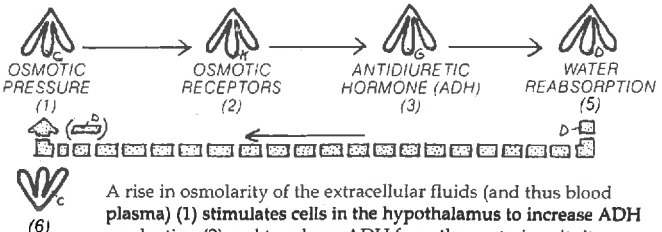




ARTERY
CAPILLARY
RECEPTOR$_K$
NEURON$_L$
ADH$_G$



NORMAL OS$_{DC}$



A rise in osmolarity of the extracellular fluids (and thus blood plasma) (1) stimulates cells in the hypothalamus to increase ADH production (2) and to release ADH from the posterior pituitary (3). ADH travels via the bloodstream (4) to the kidney (5), where it promotes water retention to relieve the rise in osmolarity (6).

THE COUNTER-CURRENT MULTIPLIER IN THE LOOP OF HENLE

The kidney regulates the internal environment by judicious excretion of ions and water. It also excretes waste products, the most notable being *urea.* Urea is produced in the liver and contains the nitrogen derived from amino acids or proteins. When these compounds are broken down by metabolism, they yield ammonia. Free ammonia is very soluble in water and very toxic. Fortunately, the liver quickly converts it to the relatively harmless urea. Metabolism of protein produces about 30 g of urea per day, which is excreted in the urine. Because ions and urea are water soluble, their excretion necessarily draws water with them. Excretion of water in the urine is obligatory, and it behooves the kidney to conserve water whenever it is in short supply by excreting a concentrated urine. What do we mean by "concentrated" urine?

TOTAL OSMOTIC CONCENTRATION IS MEASURED IN MILLIOSMOLES

Ordinarily, we express the concentration of a solute like urea by the number of moles (1 mole = 6×10^{23} molecules) of urea contained in each liter of solution. This is the *molar concentration* of urea. When this number is small, we reduce the unit by 1000 and call it a millimole (mM; 1000 millimoles = 1 mole). In every solution, each specific solute has its own molar (or millimolar) concentration. When we are dealing with osmotic water movements, all molecules and ions make an almost equal contribution to osmotic pressures. A 100 mM solution of urea exerts the same osmotic force as a 100 mM glucose solution because they contain the same number of molecules per liter. A solution containing both (100 mM urea + 100 mM glucose) contains twice as many molecules per liter and exerts twice as much osmotic force. The sum of the molar concentration of all the molecules and ions in a given solution is called the *osmolar* concentration (Osm). Sometimes we use *milliOsmolar* (mOsm) instead (1000 mOsm = 1 Osm). The "total solute concentration" of a solution containing 100 mM urea + 100 mM glucose is 200 mOsm. (Note that 100 mM NaCl would be 200 mOsm because it contains 100 mM Na^+ + 100 mM Cl^-.) The total concentration of blood plasma is consistently about 300 mOsm: urine is commonly around 950 but can range from 50 to 1400 mOsm.

CONCENTRATION OF INTERSTITIAL FLUID IN THE LOWER MEDULLA CAN BE AS HIGH AS 1400 MOSM

Na^+ pumps in the loop of Henle create gradients of only 200 mOsm — Excretion of concentrated urine requires an *interstitial fluid space* in the *medulla* 4 to 4.5 times more concentrated (1200 to 1400 mOsm) than blood plasma. To create this space, the kidneys rely on Na^+ pumps in the *ascending loop of Henle* that create only 200 mOsm gradients across the tubular cells. Because proximal tubule fluid delivered to the loop is isotonic (300 mOsm), the most concentrated interstitial space possible should be about 500 mOsm. How does the kidney manage to get 1400 mOsm?

The ability of the Na^+ pump to create a 200 mOsm gradient is called the *"single effect."* The single effect is multiplied several-fold by imbedding the pumps in the ascending limb of the two streams moving in opposite directions *(counter-current)* through the loop of Henle.

The descending limbs deliver concentration boosts to pumps in the ascending limbs — The ascending limb is impermeable to water; NaCl is pumped out into the interstitial fluid *(ISF)*, but water cannot follow. The NaCl that has been pumped creates a small gradient, 200 mOsm, so the ISF becomes slightly hypertonic. The descending limb is permeable to both NaCl and water; NaCl diffuses down its concentration gradient from the ISF into the descending limb, while water is drawn out of the descending limb into the hypertonic environment. This loss of water and gain of solute makes the contents of the descending limb hypertonic, like the ISF. But the slightly concentrated fluid in the descending limb moves! It flows toward the ascending limb, where pumps create the same 200 mOsm gradient — only this time they begin with a higher concentration and create a correspondingly higher concentration in the ISF. The cycle repeats, with elevated concentrations delivered to the descending limb, which in turn delivers these elevated concentrations to pumps in the ascending limb; *the single effect is multiplied.* The concentration of solutes in the ISF builds up until a steady state is reached, whereby amounts delivered to the ISF are just balanced by the amounts taken away by the blood supply.

The diagram on the right illustrates the scheme in a steady state. Note that the proximal tubule continues to deliver isotonic fluid (300 mOsm) to the loop, but as it descends, the fluid becomes more concentrated as NaCl enters and water leaves. The greatest concentration is at the tip. Upon ascending, the fluid becomes less concentrated as NaCl is pumped out without any water. Finally, fluid leaves the loop less concentrated (100 mOsm) than when it came in. Because the ascending limb is impermeable to water, relatively more NaCl than water is left behind in the medullary ISF.

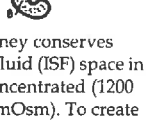
UREA'S IMPORTANT CONTRIBUTION TO MEDULLARY ISF

In addition to its effect on water permeability, ADH also increases the urea permeability of the lower medullary collecting duct. With ADH present, urea makes a substantial contribution to the ISF solute concentration in the medulla. Urea becomes trapped in the lower medullary ISF as it flows in a circle along the following path (lower left illustration): lower collecting duct → lower medullary ISF → thin ascending limb → thick ascending limb → distal tubule → collecting duct → lower collecting duct → ... This circulation and trapping occurs because the upper portions of the collecting duct are impermeable to urea, and as water is reabsorbed, the remaining urea becomes concentrated. When it reaches the lower portions, the collecting duct becomes permeable (ADH) and urea diffuses to the ISF. From there, some of it diffuses into the lower thin ascending limb, which is urea permeable. The thick ascending limb and distal tubule are not permeable to urea. As water is withdrawn from these portions, the urea becomes even more concentrated, only to be delivered to the collecting duct, where the cycle begins anew. In this way, the urea circulates and becomes more and more concentrated in all sections of its route (including the ISF) until it reaches a steady state where the delivery of "new" urea is just balanced by the amounts of urea the blood circulation carries away.

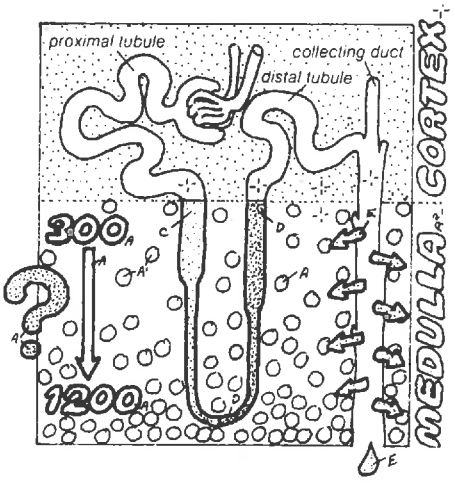
CN: Use light blue for E, darker colors for B, C, and D.
1. Begin by coloring the entire title in the upper left corner, noting that the NaCl pump (B) receives a different color. Color the anatomical drawing of the loop of Henle.
2. Color the counter-current multiplier by starting in the upper left corner with the NaCl solutes (A) entering the descending limb. Work your way down the limb, coloring the numbers and solutes in the interstitial fluid and the diffusion/gradient symbol (A) moving into the descending limb. The broken line suggests that the membranes of the cells of descending limbs are permeable to water (E) (arrows entering ISF). Then work your way up the ascending limb.
3. Color the way in which urea becomes trapped in the ISF. The drawing of the ascending limb of Henle, distal tubule, and collecting duct is highly simplified. Note that here the water-permeable membranes (broken lines) are not colored.

PROBLEM:

HOW TO CREATE A 300 - 1200 mOsm CONCENTRATION GRADIENT IN THE MEDULLA WITH ONLY A 200 mOsm $NaCl_B$ PUMP?



By excreting concentrated urine, the kidney conserves body water. This requires an interstitial fluid (ISF) space in the medulla some 4 to 4.5 times more concentrated (1200 to 1400 mOsm) than blood plasma (300 mOsm). To create this space, the kidneys rely on Na^+ pumps that create only 200 mOsm gradients across the cells. Since proximal tubule fluid delivered to the loop is isotonic (300 mOsm), it would appear that the most concentrated ISF is 500 mOsm. How does the kidney manage to get 1200 mOsm?

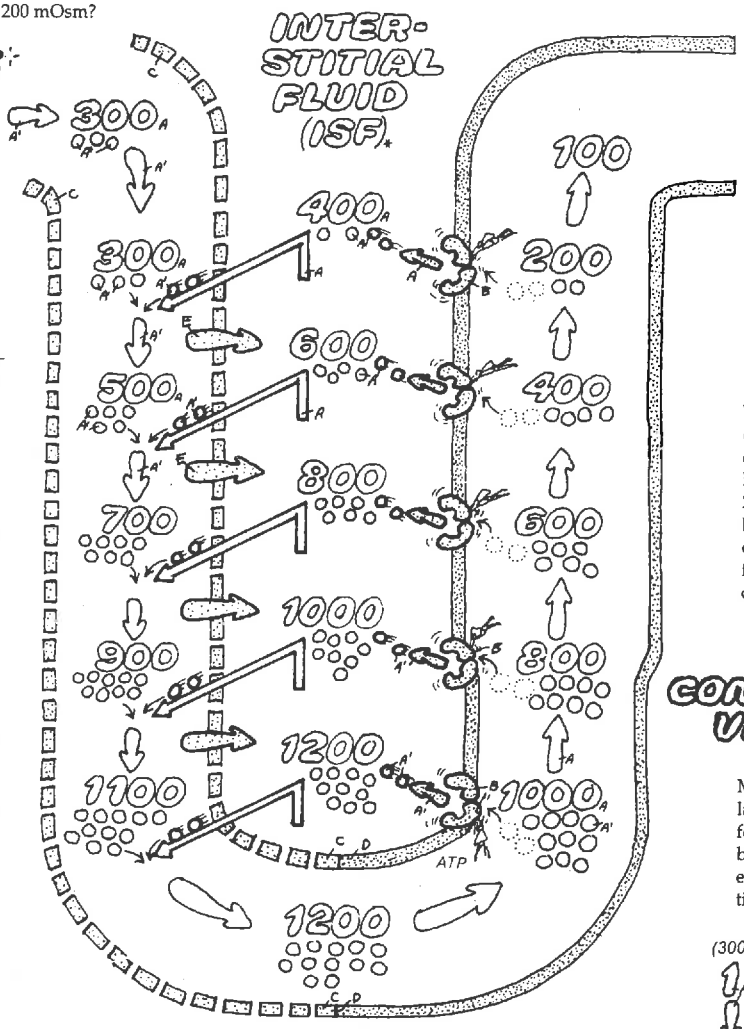


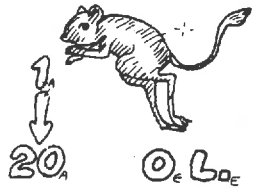
LOOP OF HENLE
DESCENDING LIMB$_C$
ASCENDING LIMB$_D$
NaCl SOLUTE$_A$
WATER$_E$

SOLUTION:

THE COUNTER-CURRENT MULTIPLIER$_A$

The ability of the Na^+ pump to create a 200 mOsm gradient is multiplied several-fold by embedding the pumps in the ascending limb of the two streams moving in opposite directions (counter-current) through the loop of Henle. The ascending limb is impermeable to water. NaCl is pumped out into the ISF, creating a small gradient (200 mOsm) and making the ISF hypertonic. The descending limb is permeable to both NaCl and water. They equilibrate passively so that the contents of the descending limb match the ISF. But the slightly concentrated fluid in the descending limb moves! It flows toward the ascending limb, where the pumps are located, giving the pumps an opportunity to create the same 200 mOsm gradient – only this time they begin with a higher concentration and can create a correspondingly higher concentration in the ISF. The cycle repeats, with elevated concentrations delivered to the descending limb, which in turn delivers these elevated concentrations to pumps in the ascending limb, the single effect is multiplied. The diagram on the right illustrates the scheme in a steady state. Note that the loop continues to receive isotonic fluid (300 mOsm), but as fluid descends it becomes more concentrated as NaCl enters and water leaves. The greatest concentration is at the bend. Upon ascending, the fluid becomes less concentrated because NaCl is pumped out without water. Finally, fluid leaves the loop less concentrated (100 mOsm) than when it came in. Relatively more NaCl than water is left behind in the medullary ISF because the ascending limb is impermeable to water. (Note: the diagram has been simplified. Pumps are actually located exclusively in the thick portion of the ascending limb).



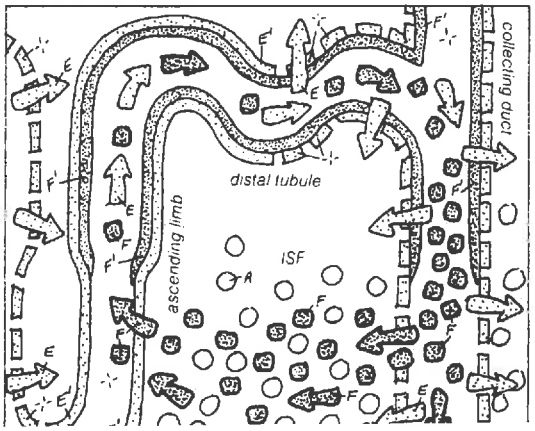


The desert rat does not need to drink water because its counter-current multiplier can establish a hypertonic medullary ISF that is 20 times more concentrated than blood plasma. Its urine is so concentrated that it can maintain body fluids with water obtained from carbohydrate breakdown.

ISF CONCENTRATION VARIATIONS$_A$

Man is able to establish a medullary ISF concentration that is only four times as concentrated as blood plasma. Therefore, the highest urine concentration is also four times that of than blood plasma.

1 L$_{DE}$
DAILY FLUID INTAKE$_E$



UREA TRAPPING$_F$

H_2O BARRIER$_{E'}$

UREA BARRIER$_F$

In the presence of ADH, urea is trapped in the ISF and contributes to its solute concentration. Follow the route taken by urea as it circulates from the collecting duct through the ISF to the ascending limb distal tubule, then back again to the collecting duct. Note places that are impermeable to urea and where water is reabsorbed, concentrating the urea.