The ability of autoregulation is persisting even following denervation (renal transplantation), indicating that neural mechanism does not play an important role in renal blood flow regulation. Mechanisms of autoregulation fail only in conditions where the blood pressure is extremely low occurring in massive bleeding when the renal blood flow is considerably restricted. Global blood flow falls also in anatomic restriction of vessel bed, e.g. in chronic renal disease and in all renal diseases connected with shrinkage of renal parenchyma.

4.4 The urine formation

Urine formation is a very complex process including glomerular ultrafiltration, tubular resorption and excretion (see figure 4.5, page 267).

4.4.1 Glomerular filtration

180l of plasma is daily filtered through glomerular capillaries and 120ml of ultrafiltrate is produced each minute. The glomerular filter is permeable for substances in a manner as if it would be provided by pores with diameter measuring 10 nm. Substances with molecular weight under 70 000 daltons appear in glomerular filtrate depending on the molecular configuration. The plasmic globulines with molecular weight of about 90 000 daltons are not at all filtered, but few amounts of albumines (m.w. 69 000 daltons) is filtered and reabsorbed in proximal tubules. Glomerular filtration does not differ substantially from filtration process occurring at arterial end of capillaries anywhere in the body. The driving force of ultrafiltration is the hydrostatic pressure of blood ensured by heart action. Ultrafiltration of blood plasma does not require local energy supply. In comparison with capillaries in striated muscles is the permeability of glomerular capillaries 400 times greater and the pressure considerably higher. The resulting effective filtering pressure is given by the difference between hydrostatic blood pressure, the oncotic pressure of plasmic proteins and the pressure inside the Bowmans capsule. The effective filtrations pressure is about 3.3kPa (25torr). The ultrafiltrate (primary urine) contains few proteins (maximally 150mg/l), non-electrolyte substances with low molecular weight (glucose, aminoacids, urea etc.), monovalent ions (sodium, potassium, chlorides etc.) approximately in the same concentration as in plasma. As the proteins of plasma are not diffusible, the rules of Gibbs-Donnans distribution governs the process of monovalent diffusible ions distribution. Small differences in concentration are due to Gibbs-Donnans effect, owing to which is the cation concentration in ultrafiltrate lower by about 5 per cent and the concentration of anions by about 5 per cent higher than in plasma. In severe paraproteinaemia and hyperlipidaemia is the concentration of these lowmolecular substances higher in ultrafiltrate than in plasma. The divalent ions concentration (calcium, magnesium) and the concentration of organic acids and bases, concerning their binding to the plasmic proteins, is substantially lower in ultrafiltrate than in plasma.

Should a substance pass from blood into the primordial urine it has to cross three barriers:

1. the endothelial layer with pores-fenestrations on the inner surface of glomerular capillaries
2. the basement membrane composed of three layers. The central layer is the proper filtering membrane. It consists of thin collagenous fibres arranged in a three-dimensional network inserted in a homogenous matrix provided by pores of 30 to 60nm in diameter
3. the layer of epithelial cells (podocytes) with numerous foot processes spanning the outer wall of glomerular capillaries. At the surface of podocytes is a layer of acid mucopolysaccharides (glycocalyx) filling the gaps between the podocytes. The permeability of capillaries is affected also by electric charge and the configuration of particles permeating into the ultrafiltrate.

Concerning the electrolyte and fluid metabolism is the glomerular filtration extremely important. It follows from the fact that kidney filter daily a fourfold volume of the entire body fluid, the fifteenfold volume of extracellular fluid, and sixtyfold volume of blood plasma.

Glomerular filtration is affected by following factors:
Figure 4.5: Glomerular filtration and the tubular function (from Thibodeau GA: Anatomy and Physiology, 1987)
1. changes of systemic arterial pressure or anatomically restrained blood supply for glomeruli (e.g. in renovascular diseases)

2. changes of intrarenal tissue pressure (in obstructions of ureters, in oedema of kidneys, in perinephritis)

3. changes in oncotic pressure of plasma (e.g. in dehydration)

4. reduction of filtration surface (involution of glomeruli, partial nephrectomy)

**Glomerular filtration** depends on age, stage of body hydration and on body surface. In first four days after the birth are renal functions reduced. Glomerular filtration is restricted to only 25-50 per cent of the filtration values in adults. The concentration ability, the excretion of water, NaCl, phosphates and the formation of ammonia are decreased, thus the newborn tends to have lower plasmic values of pH and bicarbonates. The tubular apparatus is less efficient and less sensitive to ADH the formation of which is reduced. In spite of restrained renal functions in sucklings and infants, they remain sufficient under normal circumstances, especially in breast-fed infants. In men is the glomerular filtration, calculated to the body surface higher. Glomerular filtration falls with age. This finding is a general information on biologic age of an individuum. Glomerular filtration varies also during the day up to 30 per cent.

### 4.4.2 Tubular functions

Changes occurring in tubules adjust the definitive volume and composition of urine so, that it complies with given conditions of organism. The role of the tubules is the excretion of useless and harmful substances and to retain the usefuls. The tubular functions are accomplished by complex processes of reabsorption and secretion (see figure 4.6, page 268).

#### 4.4.2.1 Mechanisms of tubular absorption

Absorption of filtered proteins is performed by pinocytosis. Protein molecules enter the tubular cells through cellular membrane. At the cell surface there are stereospecific receptors capable to catch a part of filtered proteins. Other substances pass by passive diffusion in direction of chemical and electrical gradients and by active transport against the chemical and electric gradients. There is oxygen consumption dur-
4.4. The urine formation

The active transport (aerobic oxidation). Both, the active and the passive transports are dependent on presence of carriers.

The majority of solutes occurring in ultrafiltrate and being absorbed in tubules are strong or weak electrically charged electrolytes. In absorption of these substances their size and the transtubular electrical potential play an important role.

Water, chlorides and urea moving passively in direction of electrical gradient without need of immediate energy supply.

Actively absorbed are: Na⁺, K⁺, PO₄⁻, amino-acids, glucose, creatine, sulphate, uric acid and ketonic substances. Existence of different transporting systems for single aminoacids was found.

4.4.2.2 Mechanisms of tubular secretion

Renal transport of substances does not occur only from tubular fluid into the interstitium. During the complex excretion process of substances from the body occurs in kidneys also the transport of substances from peritubular fluid into the tubules. This process is termed tubular secretion. Tubular secretion participates substantially in regulation of acid-base balance and in maintenance of isotonia.

The tubular secretion can be accomplished:

1. actively with limited transport maximum (Tm)
2. actively with transport capacity limited by gradient and time
3. passively

The active mechanisms limited by Tm or by gradient and time need energy supply for substance transport from blood into the urine. The passive secretion is accomplished without direct energy supply, but the concentration and electric gradients are dependent on energy supply. Profoundly investigated is the mechanism with limited Tm in case of paraaminohippuric acid (PAH) excretion. Its excretion by glomerular filtration and tubular secretion raises proportionally with elevation of its concentration in plasma. By exceeding a certain level remains the tubular secretion constant and independent of plasmatic concentration. For that reason is PAH clearance used for determination of renal blood flow.

By tubular secretion come weak acids and bases (ammonia, salicylic acid, phenobarbital, penicillin etc.) and potassium into the urine. Hydrogen ions are secreted by active mechanism exhibiting limitation by gradient and probably by time. In proximal tubule occurs the secretion of hydrogen ions in large amount against a low concentration gradient, and in collecting tubules it occurs in small amount against a high concentration gradient.

4.4.2.3 Maintenance of extracellular milieu stability

Kidneys maintain the stability of extracellular milieu, creating so optimal conditions for the intracellular milieu.

The sodium amount determinates a priori the volume of extracellular fluid, because it represents 80 to 90% of osmotic active substances in plasma and in interstitial fluid.

Kidneys perform, in this respect, an irreplaceable function in excreting the sodium excess and, on the contrary, if it is needed, retaining it in the body. This function is accomplished in cooperation with endocrine system by mineralocorticoids, produced in suprarenal cortex. The main “representative” of mineralocorticoids is aldosterone ensuring the sodium economy in kidneys and by it the body fluid volume regulation.

The aldosterone receptors are found in several cells along the entire nephron. The maximal number of aldosterone receptors occur probably in cells of collecting tubules. Aldosterone stimulates in collecting tubules the sodium reabsorption and the potassium excretion. It is named: the aldosterone dependent sodium resorption and potassium excretion.

A further important hormonal system afecting the renal functions is the kallikrein-kinin system. Kallikrein is produced in cells of distal tubules, where the highest activity of kallikrein-synthetase has been found. Kallikrein converts plasmatic kininogens into kinins (bradykinin, lysyl-bradykinin, methionyl-lysyl-brady-kinin) by proteolysis. It is interesting, that the same enzyme converting angiotensin I to angiotensin II desintegrates also the kinins. Generally is the effect of kinins opposite to the angiotensin effect. Kinins are potent vasodilators i and in kidneys
they stimulate the prostaglandin release (PGE$_2$). They reduce the vasoconstrictive and antidiuretic action of angiotensin. Kinin dilate vas afferens and vas efferens.

Sodium secretion is affected by glucocorticoids, namely the cortisol, (but also by progesterone). *Glucocorticoids* enhance the sodium reabsorption and, to the contrary to mineralocorticoids, they increase the glomerular filtration. The glomerular ultrafiltrate volume diminishes in absence of glucocorticoids and under the water loading the excretion of excessive water is decreased, owing to the enhanced permeability of distal tubules to water. Progesterone is an antagonist of tubular effects of aldosterone. It increases the sodium excretion by spirolactone mechanism. It reduces the aldosterone effect directly in renal tubules.

**Sodium reabsorption** with its accompanying ion (mainly Cl$^-$) occurs in proximal tubule followed by water since epithelium is permeable to water. Sodium salts represent 80 percent of solutes in ultrafiltrate. In addition, the sodium reabsorption functions as pumping system for transport of other substances. About 60 to 75 percent of sodium filtered in proximal tubule is reabsorbed with accompanying ions, chiefly with chlorides and bicarbonates. It is an isoosmotic process occurring with equivalent volume of water. Thus, the tubular fluid is in the entire proximal tubule isonatraemic and isoosmotic with plasma (sodium concentration equals 140 mmol/l, osmolality about 300 mosm/l). If poorly resorbable but osmotically active substances, e.g. mannitol enter the tubular fluid, the sodium and water reabsorption continues, but a part of water is retained in the tubular fluid to keep this fluid isoosmotic with the interstitial fluid. By this way can be the sodium concentration in tubular fluid reduced even to one half.

According the **filtered sodium load**, concomitantly occurs its reabsorption in tubules. During larger changes of glomerular filtration this compensation usually not sufficient (glomerulo-tubular dissociation). As mentioned above, sodium is transported passively from tubules independently of metabolic energy, its active transport is dependent of energy supply. The cells of proximal tubules are functionally polarized: the permeability of luminal membrane portion is more permeable to the passive sodium flow from the tubular lumen into the cell. The portion of cell membrane oriented towards the interstitium and intracellular space has a lower permeability. Here actually occurs the active sodium transport by sodium pump.

**Active transtubular sodium transport** begins with passive sodium transport across the luminal membrane of proximal tubule cells, owing to the electrochemical gradient between tubule and tubular cell. Sodium is transported alone or with HCO$_3^-$ anion, or in cotransport with aminoacids phosphates, glucose, uric acid and other substances. The cotransport with sodium enables some substances to cross the luminal membrane even against the concentration gradient leading to their increased cellular concentration. They move subsequently in direction of concentration gradient by passive transport entering the peritubular space and the peritubular capillaries. The decreasing Na$^+$ gradient between the tubule and the tubular cell is caused by sodium pump, the enzyme Na$^+$–K$^+$–ATP-ase, converting the high-energy binding of anorganic phosphorus in ATP to electrochemical gradient. By the hydrolysis of one ATP molecule three Na$^+$ are transported from the cell and two K$^+$ into the cell. So a low cellular sodium ion concentration is maintained in the cell in comparison with the sodium concentration in tubule (chemical gradient) and high concentration of K$^+$ which flows from the cell according its chemical gradient. Owing to this, becomes the cell inside electronegative against its surroundings (-70 to -90 mV).

By the transtubular sodium and relevant anion transport (Cl$^-$, HCO$_3^-$, aminoacids, etc.) into the intercellular space and the peritubular labyrinth the so called stationary osmotic gradient is created. But, it is immediately disturbed by retrograde diffusion through intracellular space into the tubule and by water diffusion into the peritubular space. Following the primary active solute reabsorption an appropriate volume of water is reabsorbed, hence the osmotic concentration in tubule or in peritubular space does not change. The hydrostatic pressure rises in peritubular space owing to the water accumulation. The fluid is absorbed from the peritubular space into the peritubular capillaries. This process depends on the hydrostatic and the colloidal osmotic pressure ratio.

The filtered potassium is almost completely reabsorbed in proximal tubule. Proximal tubule is, in addition, the site of active resorption of other substances (calcium, magnesium, glucose, aminoacids etc.) and from pharmacological point of view the
4.4. The urine formation

Calcium reabsorption occurs at the same sites as the sodium reabsorption: mainly in the proximal tubule. About a half of plasmatic calcium only is soluble and filtrable, the rest is bound to plasmatic proteins. In ultrafiltrate and in plasma an equal ratio between calcium and sodium ions is maintained.

Total plasmatic calcium concentration is 2.25 to 2.75 mmol/l. A part of it is bound to proteins, another part is present in diffusible form as free Ca^{2+} ions and its compounds like phosphates, citrates and bicarbonates. About 0.5 to 5.0 per cent of filtered calcium loading is excreted by urine. Tubular reabsorption depends on filtered calcium loading. Calcium is actively transported across the peritubular membrane by Ca^{2+}–Na^{+}–ATP-ase. Parathormone enhances the reabsorption in the distal tubule. Thyreocalcitonin and cortisol decrease the calcium reabsorption. Parathyroidectomy leads to enhanced calcium excretion by urine. Thyroxine stimulating the bone tissue metabolism induces hypercalcuria.

Under physiological circumstances about 90 per cent of filtered phosphate is reabsorbed in proximal tubules. Parathormone enhances phosphate excretion by inhibition of its reabsorption. Increased parathormone secretion may cause that less than 15 per cent of phosphates is reabsorbed. Optimal phosphate excretion is achieved by progressive reduction of phosphate reabsorption in proximal tubules. Rise in parathormone release during decrease in nephron number is the major regulating factor of phosphate excretion. Parathyroidectomy leads to enhanced calcium excretion by urine. Thyroxine stimulating the bone tissue metabolism induces hypercalcuria.

Kidneys play an important role in conversion of vitamin D to its active metabolites. The precur-
$U_g$ are the glucose concentrations in plasma and in urine, and $V$ is the urine volume per minute.

$T_{mg}$ provides ionformation about the total functional capacity of tubular cells to transport glucose. The determination of $T_{mg}$ is technically rather difficult because such a glucose concentration has to be attained and maintained which leads to glycosuria. Renal threshold is changing indirectly with the glomerular filtration and directly with $T_{mg}$.

There is a linear relation between the **glucose transport and sodium reabsorption** in proximal tubule. This relation can be observed also in reabsorption of some aminoacids. The sodium reabsorption in proximal tubule depends more upon the glomerular filtration than on reabsorption of glucose. In the case when the tubular sodium load is larger, the proximal tubule reabsorbes more sodium. As mentioned above, the reabsorption capacity of tubules is so large, that the tubular load must exceed three times the filtrated load to saturate the tubular reabsorption capacity to such a degree, that the excess of glucose could leak into the urine. The transport maximum of glucose depends partly of sodium and water reabsorption. As the glomerular filtration increases, so raises the sodium, water and glucose reabsorption. But, if the tubular glucose load markedly rises owing to the high plasmatic glucose level, its reabsorption increases considerably, even in comparison with water and sodium reabsorption. But, if the $T_{mg}$ is attained, the glucose excretion into the urine begins. The mechanism of glucose excretion is not well understood. It could be very probably performed by active transport. The fact that cyanides, acidosis and hypoxia inhibit the glucose transport might be evidence of this assumption.

Kidneys play an important role in the **uric acid** excretion although the uric acid constitutes only 5 per cent of the global amount of nitrogenous substances metabolism products. The uric acid excretion is especially important in hyperuricaemic syndrome. Uric acid is freely filtered in glomeruli like other organic acids. In addition it is secreted in proximal tubule and further partly reabsorbed. In comparison with inulin and creatinine clearance is the clearance of uric acid low, only 12 ml/min. (clearance of creatinine is about 120 ml/min.). Of practical importance is the fact, that undissociated uric acid is poorly soluble. The solubility of uric acid is reduced by acidosis. At pH 7.4 is 98 per cent of uric acid in dissociated form. If the pH falls up to 4.7 only 5 per cent of uric acid is dissociated. In these conditions may the uric acid undergo the process of crystal nucleation and urate stones formation. Urate crystals (calculi) are formed from uric acid easier when the sodium concentration is higher. The uric acid precipitation can be prevented and inhibited by water diuresis.

The most important end-product of protein metabolism is the **urea**. Urea is per se not very toxic for the organism. During uraemia is its level an indicator of concomitantly retained toxic products of metabolism. Its plasmatic level is usually not stable, depending on food protein intake and its metabolic turnover. Under normal circumstances can the plasmatic urea level vary from physiologic value (4,5 mol/l) up to twice higher values. Clearance of urea in healthy subjects is about 50 to 60 per cent of filtered urea amount. The clearance of urea much more than the inulin clearance. For that reason can the urea clearance be an approximate indicator of renal function disturbances. Nevertheless, it does not enable to discern between the haemodynamically caused disorders and renal disturbances. The urea reabsorption is considered to be a passive process and is explained by the mechanism of retrograde diffusion or by solvent drag. The urea reabsorption depends not only of its concentration in tubular fluid and of transtubular retrograde water diffusion, but also of tubular permeability to urea and of tubular fluid volume. The major factor is probably the concentration gradient of urea between the tubular fluid and surrounding interstitium. If the membrane of tubular cells is intact, urea diffuses isoosmotically together with water. The same mechanism works probably also with other substances.

**4.4.2.4 Na\(^{+}\) and Cl\(^{-}\) reabsorption in the loop of Henle by counter-current multiplication system**

In the process of urine concentration participate, above all, the descending and ascending limbs of Henle’s loop, the collecting tubule and vasa recta. The driving force of the urine concentration process is the active sodium transport from the ascending
4.4. The urine formation

Figure 4.7: Bicarbonate exchange

limb of Henle’s loop into the surrounding interstitium. Because this segment of nephron is impermeable to water, water can not diffuse with sodium. The descending limb of Henle’s loop is in presence of ADH permeable to water. Provided the concentration gradient is present, water is sucked up into the interstitium. The osmotic gradient between the ascending and descending limbs of Henle’s loop resp. the interstitium may constitute only a small part of the concentration gradient between the plasma and the definitive urine providing it operates along the entire loop of Henle. The tubular fluid is flowing namely in both limbs of Henle’s loop in opposite direction, thus the water diffuses permanently from the ascending limb of Henle’s loop into the interstitium being always little more hyperosmotic. This is maintained despite the low osmotic gradient owing to the permanent water diffusion and a progressive increase of tubular fluid osmolality towards the papilla. On the contrary, the fluid flowing in the ascending limb of Henle’s loop has always a little higher osmolality than the surrounding interstitium. This fact supports (stimulates) sodium transport into the interstitium. Many of these partial effects are multified in the counter-current system, thus a concentration difference arises increasing gradually towards the bend of the loop and attaining multiple values of single partial effects. From thermodynamic point of view this is a process of extraordinary economy, requiring minimum of energy in comparison with the energy amount which would kidneys expend during the process of the urine concentration to its definitive concentration performed in a single step.

At the end of ascending limb of Henle’s loop becomes the fluid again hypsosmotic, its sodium concentration being in comparison with the plasmatic concentration (140 mmol/l) by 100 mmol/l lower. This fluid enters the distal tube with its wall again permeable to water, enabling to attain the osmotic balance, that means, equilibrating the osmotic concentration gradients at the end of the distal tubule between the tubular fluid and the isomotic interstitium. The water diffusion and further sodium reabsorption result in decrease of tubular fluid volume by a half in comparison with the volume in loop of Henle. Tubular fluid flows then in collecting ducts towards the papilla, passing regions with rising sodium concentration in interstitium. This hyperosmotic milieu,
in presence of ADH, sucks the water from collecting tubule until the appears of osmotic balance.

In addition to the counter-current exchange system contribute also the vasa recta to diluting and concentrating functions of kidneys. As their wall is permeable to water and osmotically active substances a water transfer (shift) from the lumen into the interstitium occurs in the descending portion of these capillaries. In their ascending part move water and osmotically active substances in opposite direction. The counter-current diffusion is diminished in vasa recta when the medullar blood flow raises. This situation may be induced by application of drugs causing vasodilation in medullar vessels. This is observed also in shock when the glomerular filtration decreases but the medullar blood flow remains unchanged. Under physiological circumstances the efficiency of counter-current diffusion in vasa recta can be diminished when the osmotic concentration gradient between renal cortex and medulla becomes decreased.

The H⁺ secretion in exchange for Na⁺ occurs probably along the entire tubule. This process is extraordinary important for homeostasis and it results in bicarbonate reabsorption, in acidifying of urine and excretion of fixed anions in combination with NH₄⁺ instead of Na⁺. Tubular cells secrete hydrogen ions which are simultaneously exchanged for other cations on the luminal poles of cells. For each hydrogen ion enters one sodium ion the cells. In proximal tubules are daily about 4000 mmol H⁺ and in the distal tubule concomitantly further 500 mmol of hydrogen ions secreted. This amount of hydrogen ions is daily secreted in excess. If 60 mmol of free acids in 1,5l is excreted daily, its pH value would fall to 1,4. The pH value of urine never falls below 4,5.

From this point of view is the role of kidneys in bicarbonate resorption very important (see figure 4.7, page 273). The filtered bicarbonates are actively, almost completely, reabsorbed in proximal tubules. The bicarbonate renal threshold is identical with their plasmatic concentration, being under normal conditions 22 to 25 mmol/l. The source of hydrogen ions exchangeables for sodium ions is the carbonic acid in the tubular cell. It is formed by following reactions:

H₂O + CO₂ ⇌ H₂CO₃ ⇌ H⁺ + HCO₃⁻

Sufficient supply of hydrogen ions to the exchange reaction requires considerably higher rate of CO₂ hydration than is the rate during the spontaneous course of this reaction. The needed hydration rate is ensured in the cell by action of the enzyme carbonic anhydrase.

During the bicarbonate reabsorption is H⁺ exchanged for Na⁺. In the tubular cell Na⁺ conjugates with HCO₃⁻ and returns into the blood in form of NaHCO₃.

Hydrogen ions react in urine with HCO₃⁻ and the created H₂CO₃ is rapidly decomposed to CO₂ and water. CO₂ rediffuses from urine and is used by tubular cell for H₂CO₃ formation or is exhaled by lungs. The exchange of hydrogen ions for sodium ions continues even when the entire amount of bicarbonate is exhausted.

During metabolic processes the phosphoric acid is formed and neutralized to neutral salts. The excretion of phosphates in form of neutral salts might lead to sodium depletion and owing to this to HCO₃⁻ decrease. The hydrogen ion reacts in urine with the buffer system, mainly with Na₂HPO₄ changing in NaH₂PO₄. Na⁺ reacts in the cell again with HCO₃⁻ and increases the alkali reserve of the organism.

In absence of bicarbonate and phosphate buffers is H⁺ exchanged for Na⁺ of a neutral salt (NaCl above all). The HCl formed would acidity urine (rise the hydrogen ion concentration) to such a degree to make the further exchange impossible (the hydrogen ion transport can surmount maximally a difference of about 3 pH units). Another mechanism however begins to operate. The renal cells synthesize ammonia, mainly from aminoacids and from glutamine. Ammonia is easily soluble in lipids and therefore freely crosses the cell membrane. It enters into the tubular fluid by the mechanism of non-ionic diffusion and forms here with H⁺ ammonium NH₄⁺. The ammonium ions are poorly liposoluble, thus they do not rediffuse, but are excreted by urine. So H⁺ as well as NH₄⁺ disappear from urine and further exchange of hydrogen ion for sodium ion is again possible, as well as further NH₄⁺ diffusion into the urine. In this way the renal cells resorb a considerable amount of Na⁺ originating from neutral salts of urine and retake it into the blood in form of bicarbonate.

Anions of strong acids in tubular fluid are excreted in form of ammonium salts with concomitant sparing the sodium and bicarbonates.