Physiology of the Kidney and of Water Balance

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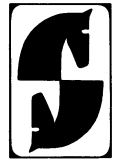

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PREFACE

This little book was assembled from the authors' lectures to medical students and was originally published as one volume in the series *Human Physiology,* edited by O. H. Gauer, K. Kramer, and R. Jung. The editors intended that each volume in this series be independent of the others and we have kept to this purpose.

We have included here only material that we feel is necessary for medical students to know in order to understand kidney function in health and, by later extrapolation, in disease. The contents rest on accepted principles established by experiments, and little space is given to what is controversial, hypothetical, or unresolved.

We are pleased that Dr. Coxon has been motivated to prepare an English language version of this text. We hope that it will serve as a ready reference and review source for the beleaguered medical student.

P. Deetjen
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INTRODUCTION

Task of the Kidney

The kidneys are the most important organs involved in the homeostatic control of the extracellular fluid, and they ensure that its volume, its osmolality, its pH, and its content of salts and other soluble substances are subjected to only the most trivial fluctuations. The kidneys exercise this control through a combination of several mechanisms. First, a certain proportion of the blood plasma is continuously filtered through the glomeruli into the tubules. As long as the salt and the water of the organism remain in balance, this ultrafiltrate (which is temporarily separated from the blood by the kidneys) is reabsorbed by the tubular cells, along with many of the substances dissolved in it, and is returned to the extracellular space. In this way a correct balance of water and electrolytes is maintained, and loss of metabolically valuable substances, such as glucose and amino acids, is prevented. If the normal composition of the extracellular fluid is disturbed by a surfeit of either water or dissolved substances, the excess is excreted.

The extracellular fluids are continuously reprocessed by the kidneys; the amount of fluid that is filtered and subjected to the modifying influence of the tubular cells every two hours is equal to the total extracellular fluid of the organism, a volume equal to about 20 percent of the body weight. In its regulatory function, the kidney is relatively independent of direct nervous control. Extra-renal influences, especially those involving the fine regulation of salt and water balance, are exerted on the kidney by humoral means, e.g., through the hormones of the adrenal cortex, the parathyroid, pituitary glands, and the kidney itself.

Morphology of the Kidney

The combined weight of the two kidneys in man is about 300 grams. The kidneys are made of 8 to 10 lobules, each consisting of a pyramidal tissue-mass, the base of which forms part of the surface of the kidney during

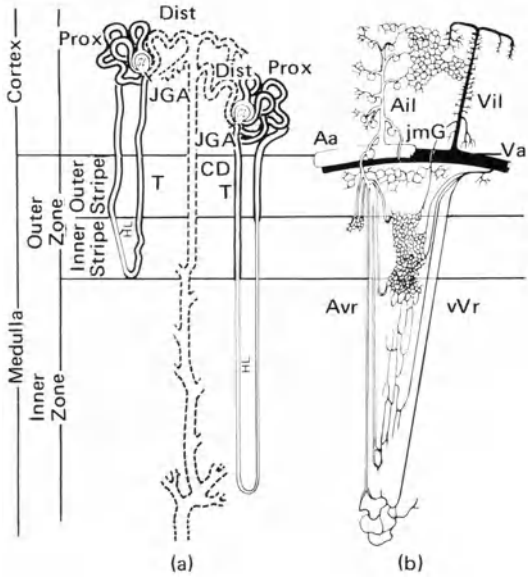


Fig. 1. The structural arrangement of a nephron and its vascular supply. On the extreme left of Fig. 1(a) is a cortical nephron and alongside it is a juxtamedullary nephron; they share a common collecting duct. The dotted lines indicate those parts of the nephron whose water permeability is increased by ADH. Gl = glomerulus; Prox = proximal convoluted tubules; HL = Henle's loop, with the thick ascending limb: JGA = juxta-glomerular apparatus; Dist = distal convoluted tubule ; CD = collecting duct; T = thick ascending limb of Henle's loop.

Figure 1(b) represents the blood vessels, labeled as follows: Aa = arcuate arteries; Ail = interlobular artery; Vil = interlobular vein; Va = arcuate vein; jmG = juxta-medullary glomerulus; aVr = arterial vasa recta; vVr = venous vasa recta. Modified from Gottschalk (33) and Moffat and Fourman (63).

fetal life. Later the lobules fuse and form a smooth, continuous contour (Fig. 1).

The human kidney closely resembles that of the dog, and this experimental animal has provided valuable information about renal physiology. In the dog, however, the individual papillae are fused into a single medullary mass. In the laboratory rat and golden hamster no lobulation is visible in the kidneys, which possess only a single, conical papilla.

In transverse section it is possible to discern in the mammalian kidney several characteristic subdivisions that comprise the cortex, and the outer and inner medullary zones, or papillae. Figure 1 illustrates how these zones relate to the disposition of the nephrons, which are themselves arranged in a regular manner parallel to one another.

The Nephron. The term "nephron" designates the smallest functional unit in the kidney. It consists of a glomerulus, a proximal convoluted tubule, Henle's loop, and a distal convoluted tubule, which opens into a collecting duct. The glomerular tuft of capillaries projects into a space called Bowman's capsule, which opens into the proximal tubule. Many nephrons discharge into one collecting duct, and in turn, several collecting ducts join as they converge on the papilla. In man each kidney comprises about 1.2 million nephrons.

As indicated in Fig. 1, glomeruli and proximal convoluted tubules are found only in the cortex and are continued into the straight portions (partes rectae) of the proximal tubules, which terminate in the outer stripe of the outer zone of the medulla. The pars recta of each tubule in fact forms the first (thick) part of Henle's loop and is continued into the thin descending portion of the loop. Those loops of Henle, which are derived from superficially located nephrons, bend back upon themselves before they reach the inner medullary zone; the ascending limb of these nephrons is lined with a thick epithelium similar to that of a proximal convoluted tubule. Nephrons whose glomeruli lie in the deeper layers of the cortex have longer Henle's loops, the extra length being contributed by the thin-walled segments of the ascending and descending limbs. A thick ascending portion of these loops begins, as in the case of the shorter cortical nephrons, at the boundary between the inner and outer zones of the medulla. Each ascending limb returns to and comes into contact with its glomerulus of origin, and at this point of contact is found the macula densa, marking the beginning of the distal convoluted tubule. The functions of each of these subdivisions of the nephron can be readily correlated with their histological differentiation.

Glomerular Filtration. An ultrafiltrate is extruded from the plasma through the walls of the glomerular capillaries.

The first application of the technique of micropuncture (see page 14) by Wearn and Richards (1924) proved that the composition of glomerular filtrate is that of an ultrafiltrate of the plasma. It is virtually free of protein, and the concentrations in it of dissolved substances of low molecular weight differ only slightly from their concentrations in plasma.

Three factors are responsible for this small but definite concentration differences between plasma and ultrafiltrate. These are (1) the volume occupied by plasma proteins, (2) the binding of weak (and certain strong) electrolytes to protein, and (3) the existence of a Gibbs-Donnan equilibrium.

Gibbs-Donnan Equilibrium. At the pH of blood, i.e., 7.4, there is an excess of negative charges on the proteins of the plasma. Furthermore, because of the presence of these nondiffusible anions, there arise slight differences between the electrolyte concentrations in plasma and in the protein-free fluid outside the capillary membranes. Note that this fluid,

having been expressed from capillaries, is a special case of extra-cellular fluid, but is intratubular instead of interstitial.

For equilibrium to exist between any interstitial fluid and plasma, two thermodynamic conditions must be fulfilled.

1. Across the capillary membrane (inside = i, outside = o), there is established an equilibrium potential (E_D). With respect to the ions to which the membrane is permeable the following relationship holds:

$$E_D = \frac{RT}{F} \ln \frac{[C^+]_i}{[C^+]_o} = \frac{RT}{F} \ln \frac{[A^-]_o}{[A^-]_i} \tag{1}$$

C^+ = cation and A^- = anion. For an explanation of the thermodynamic symbols, see pages 25-26.

This expression can be simplified to:

$$\frac{[C^+]_i}{[C^+]_o} = \frac{[A^-]_o}{[A^-]_i}$$

which is equivalent to

$$[C^+]_o \times [A^-]_o = [C^+]_i \times [A^-]_i \tag{1a}$$

Hence, at equilibrium the products of the concentrations of diffusible ions of opposite sign on the two sides of the membrane must equal one another.

2. Electrical neutrality must be preserved on each side of the capillary membrane. Thus on the outside,

$$[C^+]_o = [A^-]_o \tag{2}$$

while on the inside,

$$[C^+]_i = [A^-]_i + [\text{Proteinate}^-]_i \tag{3}$$

Thus, from Equations (1a), (2), and (3),

$$[A^-]_i \times ([A^-]_i + [\text{Proteinate}^-]_i) = ([A^-]_o)^2 \tag{4}$$

Similarly, for the cations,

$$[C^+]_i \times ([C^+]_i - [\text{Proteinate}^-]_i) = ([C^+]_o)^2 \tag{5}$$

Equation (4) may be written in the form

$$([A^-]_i)^2 + ([A^-]_i \times [\text{Proteinate}^-]_i) = ([A^-]_o)^2$$

from which it follows that (for permeating anions)

$$[A^-]_i < [A^-]_o$$

Similarly, for permeating cations, Equation (5) indicates that

$$[C^+]_i > [C^+]_o$$

By way of example consider a membrane permeable to sodium ions and chloride ions but not permeable to protein anions, placed between two equal volumes of fluid. Let there be 15 units of sodium ion on each side of the membrane, but on one side let electrical neutrality be attained by the presence of 15 units of chloride ion, and on the other side by 10 units of protein ion and 5 of chloride (see Fig. 2a). After the establishment of a Gibbs-Donnan equilibrium, the diffusible species of ion are unequally distributed across the membrane (see Fig. 2b). The first of the thermodynamic conditions is fulfilled when the product of the concentrations of diffusible ions on one side is equal to that on the other ($8 \times 18 = 144 = 12 \times 12$). The second condition is fulfilled since electrical neutrality is preserved on both sides of the membrane. Although there now exists a concentration gradient for sodium from the left to the right compartment and for chloride in the opposite direction, this unequal distribution of diffusing ions is maintained. In our example, at equilibrium there would be found in the protein-containing compartment 18 + 8 (26) units of diffusible ion, while on the other side of the membrane there would be only 12 + 12 (24) units. There is thus an imbalance in the total number of

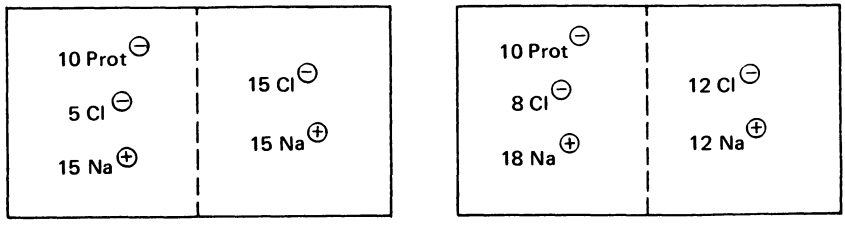


Fig. 2. Example of the establishment of a Gibbs-Donnan equilibrium across a membrane (— — — — —) that is impermeable to protein. (a) Initial conditions (b) Conditions after the Gibbs-Donnan equilibrium is set up.

diffusible ions, and this means that on the protein-containing side the concentration of osmotically active particles is greater than on the other side, even if one neglects the nondiffusible protein anions. If the volumes of the two sides were not, as in our hypothetical example, fixed, then a flux of water would occur as a result of this osmotic gradient. The volume of the protein-containing compartment would then expand at the expense of the other. The so-called oncotic pressure of the blood plasma is thus caused not only by the colloidal osmotic pressure of the protein molecules themselves, but also by the excess of the diffusible ions that arises on the protein-containing side as a result of the Gibbs-Donnan distribution.

It has already been seen in Equation (1) that the establishment of a chemical equilibrium across the membrane is accompanied by the establishment of an electrical potential difference, the so-called Donnan potential. The magnitude of this potential can be calculated from the Nernst equation. Since, as can be seen from the summary in Table 1, the differences in concentration of the important electrolytes between plasma and interstitial fluid are small, the Donnan potential that exists between the plasma and the interstitial space is correspondingly small, amounting to about 1 millivolt.

The concentration of univalent anions in protein-free interstitial fluid is set by the Gibbs-Donnan equilibrium at about 5 percent higher than in plasma water, so that the so-called Donnan factors amounts to 1.05. In the case of the univalent cations, this factor is 0.95. For the divalent cations

Table 1. Ionic composition of plasma and interstitial fluid (glomerular filtrate) based on the Gibbs-Donnan distribution. From Pitts (70).

Subtance	Plasma (meq/l.)	Plasma water (meq/l.)	Interstitial fluid (meq/l.)
Na	142	151	144
K	4	4.3	4
Ca	5	5.4	2.5
Mg	3	3.2	1.5
Cation	154	163.9	152.0
Cl	103	109.7	114
HCO_3	27	28.7	30
PO_4	2	2.1	2.0
SO_4	1	1.1	1.0
Organic acid	5	5.3	5.0
Protein	16	17	0.0
Anion	154	163.9	152.0

and anions it is not possible to specify an exact factor, since calcium and magnesium are partially bound to proteins. The total concentrations listed in Table 1 are corrected for plasma water, and represent substances free in solution as well as those bound to protein. Hence, in the case of the divalent cation, substantial contributions to the concentration differences between plasma and ultra-filtrate are a result of protein-binding.

In the case of weak electrolytes, organic acids, and bases, protein-binding can be considerably stronger, depending on the pH, protein concentration, and ligand concentration. It may amount to as much as 90 percent.

Correction Factor for Free Solvent. In a liter of plasma not all of the total volume is available to dissolve crystalline substances, because protein is present. But in the protein-free ultrafiltrate, virtually the whole volume is available for this purpose. Proteins have a higher specific gravity than water, and the proteins present in plasma occupy per gram only about 0.75 milliliter. The free-solvent correction factor can thus be calculated according to the following equation:

$$F = \frac{100}{100 - (\text{g \% plasma} \times 0{\cdot}75)} \tag{6}$$

When the average protein concentration in plasma is 6 g %, the volume correction factor, F, is equal to 1.05, and so concentration of the individual dissolved substance is thus about 5 percent higher in plasma water than in whole plasma.

In the case of the univalent cations, sodium and potassium, it is sufficient for practical purposes to regard their concentration in plasma ultrafiltrate as equal to their concentration in whole plasma, because the Donnan factor and the correction for protein volume cancel each other almost exactly.

Filtration Process. Glomerular filtration is a passive process, the driving force for which is the algebraic sum of the hydrostatic pressure (supplied by the heart) and osmotic pressure gradients across the capillary membrane. If we assume that water and solutes move across the glomerular capillary membrane through pores, pore size is the most important factor in determining the molecular size of substances that can be filtered. Table 2 shows substances arranged in order of their molecular weights and calculated molecular radii. The molecular radius is equal to approximately 0.15 times the square root of the molecular weight (27). In the last column of the table the filtration rate of the individual substance is compared with that of creatinine, which is as freely filterable as water. With increasing molecular radius the filtration rate of the substance falls. As serum albumin with a radius of about 39.4 Å has a filtration rate that is only 1 percent that

Table 2. Physical properties of molecules and their ability to pass through the capillary wall. Modified from Pappenheimer (67).

Substance	Molecular weight	Radius of equivalent sphere, Å	Shape of dimensions of main axes,[a] Å	Glomerular clearance substance: creatinine
Water	18	0.6	—	1.0
Urea	60	1.2	—	1.0
Glucose	180	2.0	—	1.0
Inulin	5500	14.8	?	0.98
Myoglobin	17,000	19.5	54 × 8 (discoid)	0.75
Egg albumin	43,500	28.5	88 × 22 (fusiform)	0.22
Hemoglobin	68,000	32.5	54 × 32 (discoid)	0.03
Serum albumin	69,000	35.5	150 × 36 (fusiform)	<0.01

[a] Based on viscosity and X-ray data.

of creatinine, the limiting molecular weight for filtration across the membrane is found to be between 80,000 and 90,000, which would correspond to a pore radius of 42 to 45 Å.

From the permeability characteristics of the glomerular membrane it is possible to calculate, by applying the Hagen-Poiseuille Law, the filtration coefficient (K_F) (78). This coefficient is also called the hydraulic conductivity of the membrane, and is then denoted by the symbol L_P. It indicates what volume of filtrate can be delivered across the membrane in question per unit time, surface, and difference in pressure. The value found for the glomerular membrane is 31×10^{-3} ml/min/mm Hg per gram of of kidney, or 300×10^{-8} ml/sec/cm^2 per centimeter of water, and is about 100 times greater than the corresponding coefficient for muscle capillaries.

In a filtration process the water flux (ϕ H_2O) across a membrane is equal to the product of the hydrostatic pressure ΔP and the filtration coefficient (K_F). Thus,

$$\phi \, H_2O = K_F \, \Delta P \tag{7}$$

Osmotic pressure difference can also promote water flux across a membrane. If the membrane is ideally semi-permeable, i.e., permeable to solvent and not at all to the solute that is creating an osmotic pressure, one may apply the following equation for dilute solutions:

$$\pi = CRT \tag{8}$$

where π is the osmotic pressure, C is the concentration of the dissolved

substance, R is the universal gas constant, and T is the absolute temperature.

Semi-permeable membranes constitute an idealized case. Biological membranes are actually more permeable to dissolved substances, and hence the osmotic pressure set up across them is correspondingly smaller. The relationship between the effective osmotic pressure and the theoretically expected osmotic pressure can be determined experimentally. This relationship is expressed by the reflection coefficient, σ (see page 46). The water flux attributable to osmotic pressure is then described by the relationship

$$\phi\ H_2O = K_F \times \sigma \times \Delta\pi \tag{9}$$

As the reflection coefficient of a particular substance approaches the upper limit of 1, the characteristics of the membrane approach ideal semi-permeability. Since in the glomerular ultrafiltrate, all plasma constituents except protein are found in concentrations almost equal to those in plasma water, only the colloidal osmotic pressure of the plasma proteins has a significant influence on the filtration process. Proteins are almost quantitatively reflected by the glomerular membrane ($\sigma \cong 1$); all other molecular solutes have a smaller reflection coefficient. Water flux through the glomerular membrane can thus be formally described as consisting of a hydrostatic component resulting from the pressure difference between the capillaries and Bowman's capsule ($P_{cap} - P_{Bow}$), this flux being opposed by a flux in the opposite direction attributable to the oncotic pressure of the plasma proteins (P_{onc}). The net water flux through the glomerular membrane, which is the glomerular filtration rate, is thus given by

$$GFR = K_F\,(P_{cap} - P_{Bow}) - K_F\,P_{onc}$$

or

$$GFR = K_F\,[P_{cap} - (P_{Bow} + P_{onc})] \tag{10}$$

The pressure difference, $P_{cap} - (P_{Bow} + P_{onc})$, has been called the *effective filtration pressure.*

For the human kidney no direct measurements exist which permit solution of the above equation, but experiments on rat kidneys allow it to be tested. P_{Bow} has been directly measured in the rat and has a mean value of 15 mm Hg; P_{onc} can be calculated for a mean protein concentration of 6.6 g %, assuming the reflection coefficient to be equal to 1. From these figures it can be computed that about 20 percent of the plasma going through the kidney at any given time is filtered, i.e., the filtration fraction is 0.2. P_{cap} has up to now been evaluated only indirectly by blocking the

outflow from the proximal tubules. A pressure of 45 to 50 mm Hg then builds up within Bowman's capsule, and this is assumed to equal $P_{cap} - P_{onc}$. During these measurements, which change the hydrostatic conditions, the pressure itself may naturally be affected. Nonetheless, if we insert these pressures into Equation (10), we obtain the following result:

$$GFR = 31 \times 10^{-3} [75 - (28 + 15)] = 1.0 \text{ ml/min/g kidney}$$

This figure agrees well with the value derived from clearance experiments used to measure the glomerular filtration rate in the rat (see below).

Structure of the Glomerular Filter. The filtering membrane has three components: a layer of capillary endothelium, basement membrane, and epithelial cells of the capsule. With the help of the electron microscope, our knowledge of the anatomy of the glomerular filter has been greatly extended. Seen under an electron microscope (see Fig. 3), the endothelial walls of capillaries are interrupted by numerous fenestrations, or pores. These fenestrations have a mean width of 500 to 1000 Å and therefore do not constitute a barrier to filtration.

On the outer surface of the capillaries is the basement membrane, and applied to this are a large number of processes that arise from specialized epithelial cells. This epithelial layer is a continuation of Bowman's capsule, which in the region of the glomerular capillaries, no longer forms a continuous sheath. Moreover, these cells are freely branching and interdigitate with each other. The peripheral ends of these numerous cytoplasmic processes are so applied to the basement membrane and capillary wall that the interstices between them are unfilled. These gaps, or "slit pores," have a breadth of between 200 to 500 Å and, like the endothelial fenestrations, would seem capable of sieving only the larger globulin molecules. Thus, there remains only the basement membrane as a possible filtration barrier. A basement membrane is always found interposed when connective tissue comes into contact with an epithelial structure. The glomerular basement membrane consists of three layers, namely, a middle layer (which is relatively electron dense) and the two more translucent layers on either side of it.

Ferritin and thorotrast molecules which have a molecular radius of about 90 Å can penetrate from the blood as far as the middle layer of the basement membrane (23). The middle layer of the basement membrane at higher resolution shows filaments of 30 to 40 Å diameter, which probably consist of collagen. They are embedded in a homogeneous matrix and are arranged in a network with a mesh size of between 30 and 75 Å. It is this filamentous network which probably constitutes the porous structure (85). It has been calculated from the behavior of substances with different molecular radii at the glomerular basement membrane that the majority of

channels inside this membrane has equivalent pore radii, varying between 2 and 45 Å, with a maximum frequency distribution at around 30 Å (27).

The pore theory of filtration is not without its opponents. An alternative theory (9) assumes that the filtrate is formed entirely by diffusion across the extent of the glomerular basement membrane. This theory has not been widely accepted, since it is difficult to imagine what electrochemical

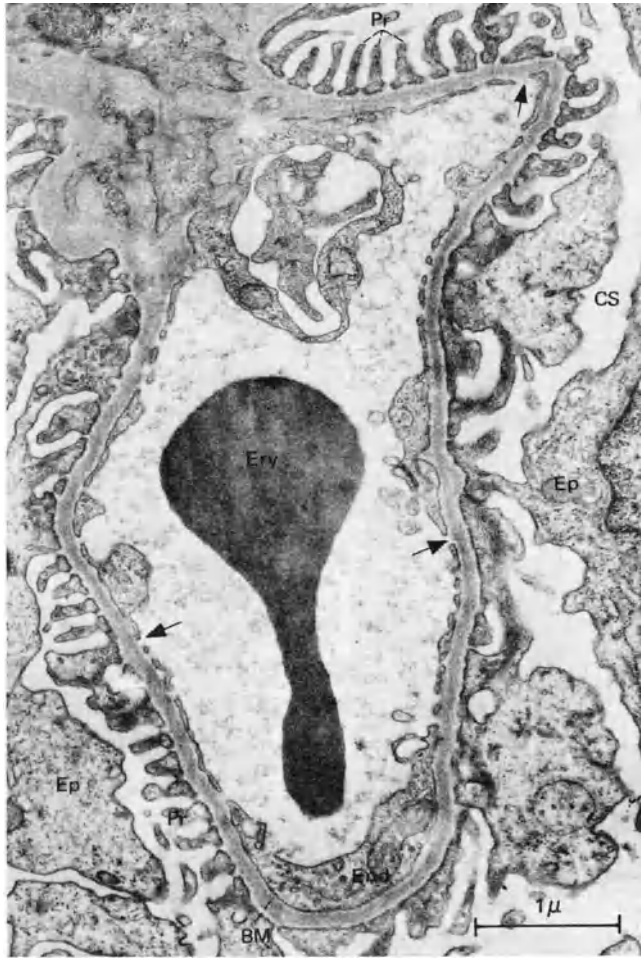


Fig. 3. A capillary loop from a glomerulus seen under the electron microscope. An erythrocyte (Ery) can be seen within the lumen. Also shown are endothelial wall (End) with pores (→); basement membrane (BM); epithelial foot processes (Pr); capsular space (CS); Ep = epithelial cell. Magnification 18,400:1. Provided by W. Thoenes.

gradients could result in net diffusion of the magnitude of the glomerular filtration rate.

Determination of the Glomerular Filtration Rate. To measure the total volume of filtrate produced by all glomeruli per unit time, it is necessary to employ an indicator substance. The ideal indicator should have the following characteristics:

1. It should be a freely filtered nonelectrolyte, i.e., it should not undergo protein binding nor should its passage through the glomerular basement membrane be impeded by its molecular size.
2. It should be neither reabsorbed nor secreted by the tubular cells. Thus, the quantity of the substance appearing in the bladder urine must be identical with that filtered at the glomeruli.
3. There should be no destruction or new formation of the substance within the kidney.
4. Finally, the substance must be free from toxic or injurious effects.

These requirements are best fulfilled by the polysaccharides inulin and polyfructosan S. When either of these substances is infused into the blood, the quantity appearing in the bladder urine per unit time is equal to that filtered in the same time interval. Since for a substance in solution:

$$\text{quantity} = \text{volume} \times \text{concentration}$$

the following equation must hold:

$$U_{\text{inulin}} \times \dot{V}_u = P_{\text{inulin}} \times \text{GFR}$$

where

U_{inulin} = inulin concentration in the urine (mg %)
P_{inulin} = inulin concentration in plasma and hence in ultrafiltrate (mg %)
$\dot{V}_u$ = urine flow (ml/min)
GFR = glomerular filtration (ml/min).

The equation states simply that the amount of inulin excreted in the urine per minute equals the amount filtered per minute.

By rearrangement of the preceding equation,

$$\text{GFR} = \frac{U_{\text{inulin}}}{P_{\text{inulin}}} \times \dot{V}_u \tag{11}$$

and has the dimensions of ml/min.

The quantities on the right-hand side of the equation can be readily measured in man, and so the GFR can be determined. The formula

$$C = \frac{U}{P} \times \dot{V}_u \tag{12}$$

has been called the "clearance formula." It defines the volume of plasma that is "cleared" of the substance in question per minute, and in the case of inulin, this volume (the clearance) is equal to the GFR. In the case of a substance such as glucose, which is also freely filterable but is normally almost completely reabsorbed by the tubules, the clearance is practically nil. Substances such as PAH (see page 58) that, as well as being filtered are also secreted by the tubular cells have a clearance that exceeds that of inulin.

Inulin and polyfructosan S are substances foreign to the body and therefore must be exogenously administered in order to measure GFR. As a consequence of the long pathway that a substance must follow from the glomeruli to the bladder, GFR will be determined more accurately as the period over which the measurements are made becomes longer; on the other hand, the longer this period becomes, the greater is the chance of the measured quantities varying during the time over which they are being measured. In order to minimize these variations, it is necessary to administer inulin, or some other indicator substance, by constant intravenous infusion so that the plasma concentration remains stable.

In common clinical practice the clearance of the endogenous substance creatinine is used as a measure of GFR instead of exogenously administered inulin. The accuracy of the so-called endogenous creatinine clearance is inferior to that of inulin because creatinine, as well as being filtered at the glomeruli, is, in man, also secreted in small amounts by the tubules. The extent of this secretion is, with the normal low concentrations (about 1 mg %) of creatinine in the plasma, usually hardly significant, although it can become so in patients with impaired kidney function, in whom the concentration of creatinine in the plasma may be elevated.

The GFR in normal adult man is around 120 ml/min and is independent of the rate of urine production. It exhibits a diurnal variation, with the daytime peak exceeding the nighttime value by some 30 percent (110). In addition, GFR varies with dietary intake, such variation having been hitherto studied principally in the dog. Figure 4 shows how, in a group of dogs, the ingestion of even a low-salt meal resulted in a 30 percent increase in GFR over the fasting value. This increase in GFR is apparently connected with the absorption of amino acids, since a similar increase can be evoked by the feeding or injection of free amino acids (45). The increase in GFR is even more marked with a high-salt diet, but disappears after a

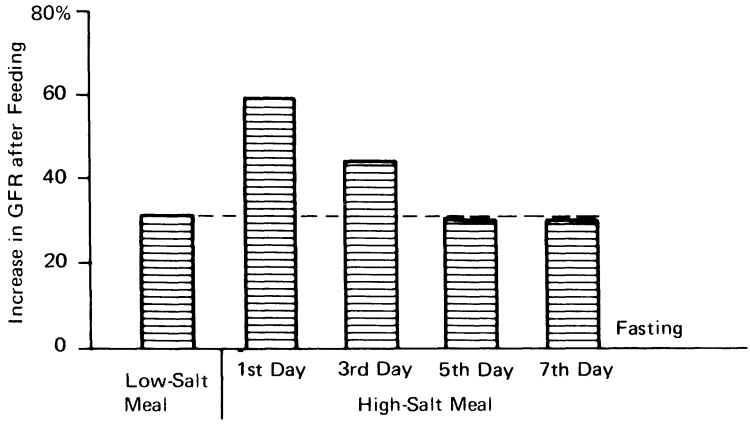


Fig. 4. Increases in GFR following a low-salt and a high-salt diet. Modified from Behrenbeck and Reinhardt (2).

few days on such a diet. After five days of a high-salt diet (see Fig. 4) GFR has usually returned to the value found on a low-salt intake.

The mechanism involved in the response of the GFR to diet or to the administration of amino acids is unknown. A possible explanation of the sodium-dependent changes in GFR is discussed on pages 34 and 125.

Tubular Transport

Micropuncture Methods. The clearance method (page 13) has the great advantage of permitting the study of renal function without operative interference with the kidney, but it has the disadvantage of giving only indirect information reflecting the overall behaviour of the nephron population. In fact there exist functional differences not only between nephrons situated in different zones of the kidney, but also between various sections of a single nephron. Therefore, in order to define localized characteristics of tubular transport, it is essential to investigate single nephrons. This is done by the technique of micropuncture, originally introduced in 1923 by Wearn and Richards (108). Since 1955 micropuncture techniques have been in increasingly general use, so that they have now become an indispensible part of the investigation of renal function.

Through the capsule of the exposed kidney of a small rodent such as the golden hamster, rat, or guinea pig, it is possible to distinguish tubular segments and peritubular capillaries, (Fig. 5) and to introduce into these, with the aid of micromanipulators, pointed glass capillary tubes having tip diameters of only a few micra.

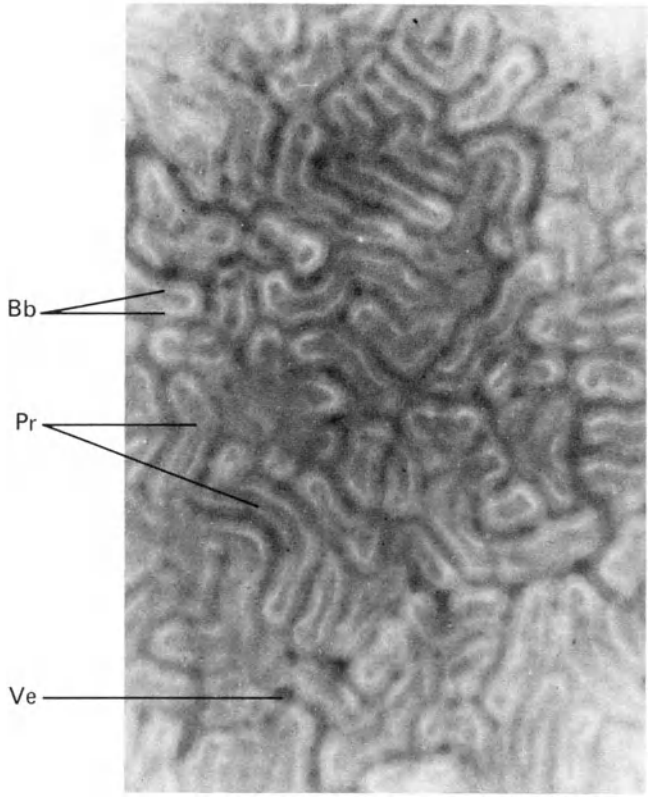


Fig. 5. Photomicrograph of the surface of a living rat kidney. Pr = convolutions of the proximal tubule; Bb = brush border; Ve = vasa efferentia branching into capillaries.

In Fig. 6 a few of the more important applications of micropuncture to the study of single nephrons are indicated schematically. The simplest type of experiment is the collection of samples of fluid from proximal and distal convolutions (both of which are accessible from the surface) and the analysis of these samples to determine the concentration of various substances. In certain desert rodents micropuncture of the loops of Henle and of collecting ducts is also possible, because in these animals the renal papilla extends deeply into the renal pelvis and can thus be exposed. By one or another of these techniques it is possible to determine the contribution of the different tubular segments to the reabsorption of filtered fluid; this is done by administering a test substance such as inulin (see page 12) and

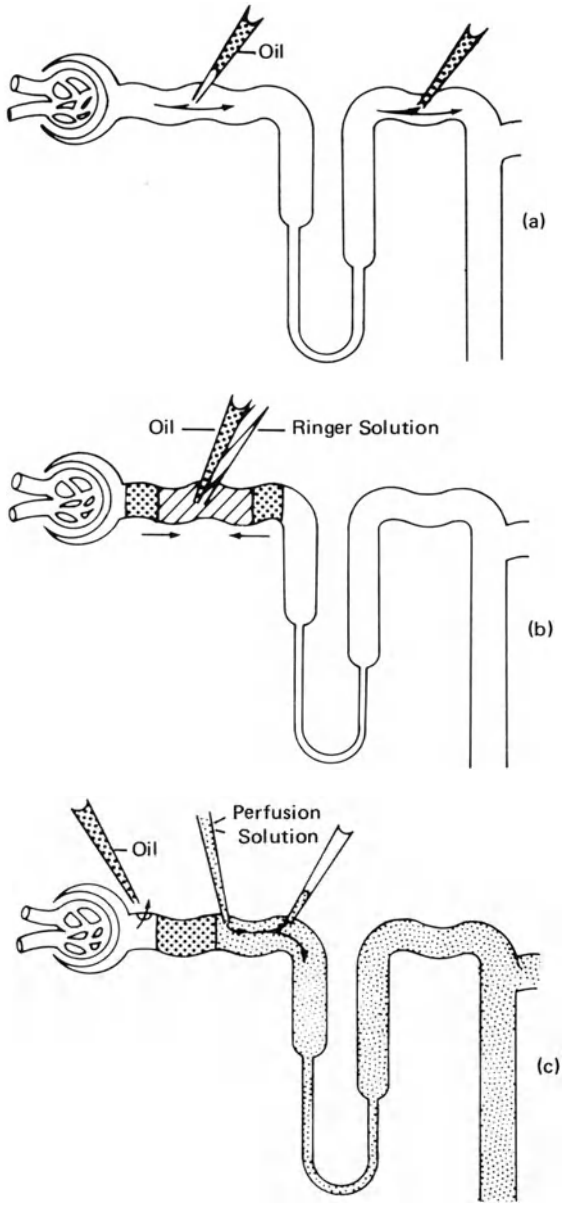


Fig. 6. Examples of some application of micropuncture: (a) collection of samples of tubular fluid from the proximal and distal convolutions under conditions of free flow; (b) use of the split oil droplet; (c) microperfusion.

measuring the increases in its concentration that occur along the length of the nephron. Since inulin is freely filterable but is not reabsorbed, the increase in its concentration is proportional to the reduction in volume of the original filtrate.

By employing a split oil droplet, it is possible to investigate the reabsorptive activity of isolated nephron segments (84). With this method a double-barreled pipette is inserted into a tubule at the surface of a rat kidney and a drop of oil is injected through one barrel into the lumen of the tubule. Then, through the second barrel a small quantity of Ringer's solution is ejected so as to split the oil drop into two portions. The tubular cells then reabsorb the Ringer solution, and the rate at which such reabsorption proceeds can be determined from the rate at which the two portions of the oil drop come together. The event is viewed through a microscope and timed with stopwatch accuracy.

In order to measure the rate of rapidly occurring transport processes, the technique of microperfusion (Figure 6(c)) was developed (89). First a small drop of oil is introduced into the stream of tubular fluid in order to establish which superficial segments belong to the same nephron. A second pipette is then inserted distal to the oil drop, and the test solution is infused through this pipette from a controlled pump. The tubular lumen between the two pipettes is now filled with oil, so that contamination with newly formed filtrate is prevented. The most distal part of the nephron can then be perfused at a predetermined rate with a test solution, and the changes produced by tubular activity can be evaluated by analysis of fluid samples taken from a third site further downstream.

The Intratubular Flow Rate

Normally 99 percent of the glomerular filtrate is reabsorbed, and only 1 percent is excreted. In the reabsorptive process the various segments of the nephron have quite different functions. By means of micropuncture (page 14) it is possible to measure the increase in inulin concentration along the tubule and so calculate what proportion of the filtered volume is reabsorbed under various conditions of urine flow. The mean results from many such measurements (94, 104, 106) are assembled in Fig. 7. Around 60 percent of the filtered volume is absorbed in the proximal tubule, and this is true whether the animal is in a state of water diuresis or antidiuresis; i.e.,whether the bladder urine is dilute or concentrated. The final volume of urine in either case is determined solely by the activity of the distal parts of the nephron. Accordingly, proximal water reabsorption is sometimes referred to as "obligatory" in contrast with distal water absorption, which is partly "facultative".

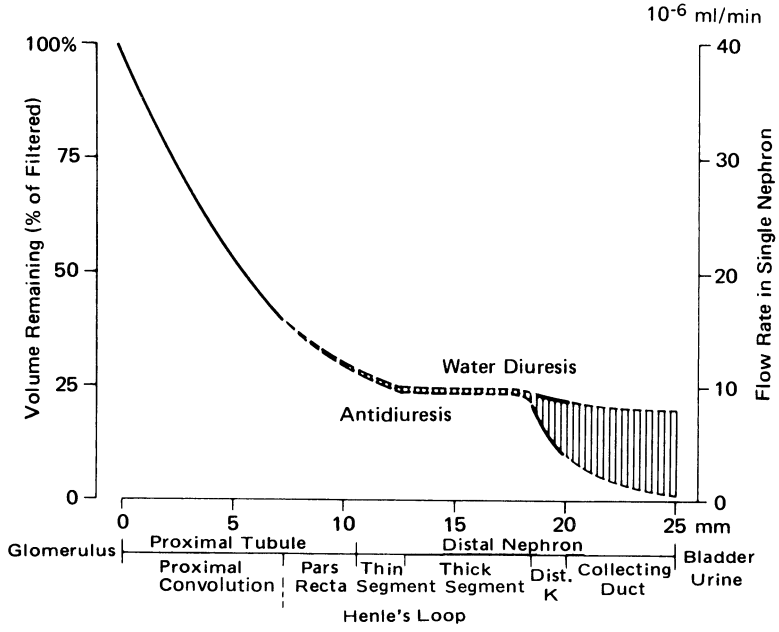


Fig. 7. Localization and magnitude of fluid reabsorption in a superficial nephron of a rat during antidiuresis and water diuresis. The percentage of filtered volume remaining in the tubular lumen was calculated from the increase in concentration of inulin. The reduction in flow rate was measured by a cinematographic method (right-hand scale). The continuous line is based on direct observations using micropuncture; the broken line represents data obtained indirectly.

In order to determine the velocity of flow in the tubules rather than the percentage reduction in volume, a micropipette is inserted into a proximal tubule as close as possible to the glomerulus, and a small quantity of concentrated dye is injected through its tip. The progress of the bolus of dye is then filmed through a microscope, and in this way the linear velocity of the dye front can be measured in its passage along the different segments of the nephron. By taking into account the cross section of these segments, determined from their measured diameters, it is possible to estimate the volume-flow rate of the tubular fluid. The decrease in the volume flow as the fluid moves distally is shown on the right-hand scale in Fig. 7.

Recently it has been established that the filtration rate is not the same in all nephrons (42). This was shown by puncturing a nephron at a selected site and collecting the fluid quantitatively. From the inulin concentration, the GFR for this individual nephron was then calculated by the usual formula. In this study it was found that the juxta-medullary nephrons (15

to 20 percent of the total population in the rat) have a filtration rate double that of the superficial cortical nephrons when the animal is fed a low-salt diet. On a high-salt diet the GFR in the cortical nephrons increase by about 60 percent, but since the juxta-medullary filtration rate is correspondingly reduced, the total GFR is almost unchanged (see Fig. 8).

It should be pointed out that while filtration proceeds at the normal rate, the tubular diameter in all regions of the kidney is between 17 and 20μ except for the thin segment of the ascending limb of the loop of Henle, where the maximum width may be only 12 μ. These measurements, however, do vary with intraluminal pressure and the flow rate of the tubular fluid. When filtration stops, the proximal tubules collapse almost completely.

Solute Transport Through the Tubular Cells

Figure 9 is a schematic drawing of a single nephron in which, according to present information, the sites and the direction (shown by arrows) of transport of a number of substances are indicated. When a substance is transported from the blood into the tubular lumen, the process is called

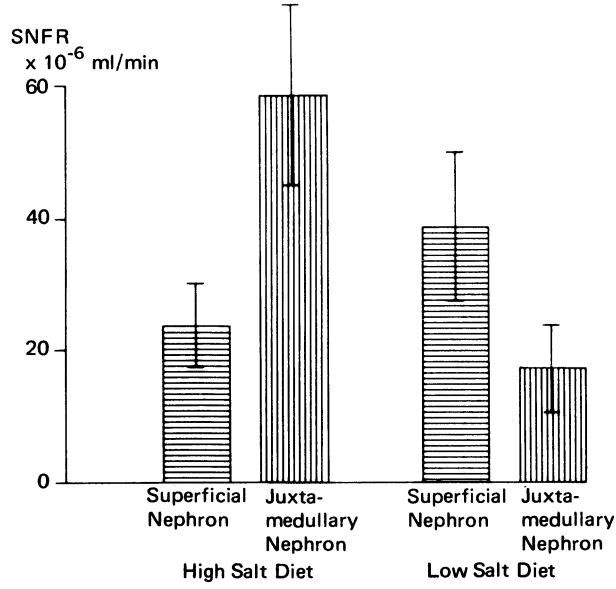


Fig. 8. The filtration rate of single nephrons (SNF) from the cortical and juxta-medullary regions of the rat kidney as affected by salt intake. The GFR of the whole kidney was 0.94 ± 0.16 ml/min on a low-salt regimen, and 1.01 ± 0.24 ml/min on a high-salt regimen. After Horster and Thurau (42).

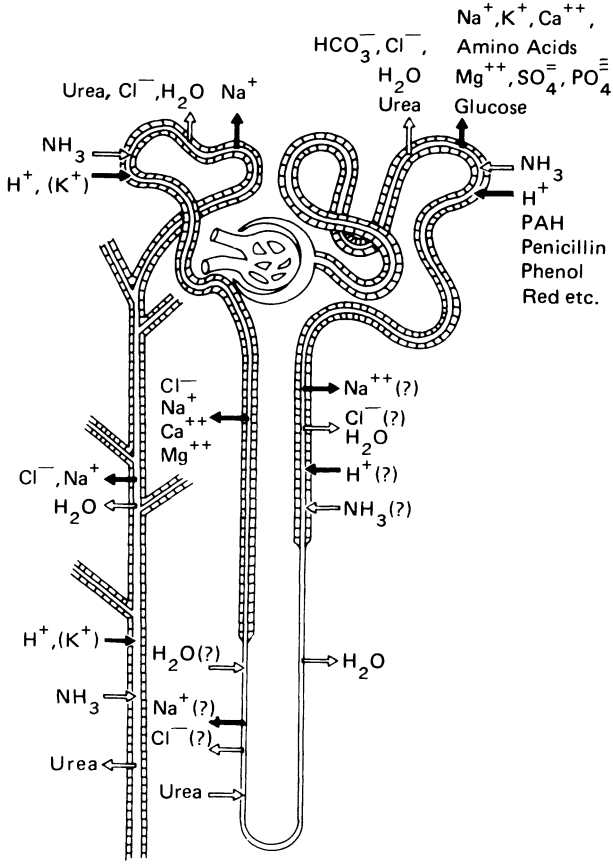


Fig. 9. Localization of tubular transport processes in the nephron. →= active transport; ⇒ = passive transport. After Thurau and Deetjen (96).

secretion. If transport occurs against an electrical or chemical gradient* and requires the use of metabolic energy, it is called *active transport* and is shown by the heavy arrows in Fig. 9.

Electrophysiology of the Nephron

As a result of rapid developments in neurophysiology during recent years, micromethods for the measurement of electrical potentials that can be applied to the tubular cells have become available (25). These reveal that

* The term "gradient" is often used in biology as a synonym for "difference," with reference to concentration, pressure, electrical potential, etc. Strictly speaking, a gradient is the first differential of the variable. Thus, in the case of a concentration gradient, the appropriate expression is dc/dx, where c = concentration and x = distance.

there exists no measurable potential difference across the wall of the proximal tubule. In the distal tubule and collecting ducts a potential difference of 40 to 50 mV has been reported (Table 3). Figure 10 shows diagrammatically the method of determining the magnitude of the potentials that can be measured in the distal tubule. If an electrode originally placed in the interstitium (Fig. 10(a)) is pushed into a distal tubular cell (Fig. 10(b)), a potential difference of 70 mV is recorded, with the interior of the cell negative with respect to the interstitium. If the electrode is advanced further so that its tip emerges into the lumen (Fig. 10(c)), 50 mV is recorded, with the lumen also negative with respect to the interstitium. Thus, a net potential difference of 20 mV exists between the

Table 3. Trans-tubular electric potential difference (E), concentration in the interstitium (I), and tubular fluid (TF) under equilibrium conditions.

	Proximal convoluted tubule	Distal convoluted tubule	Collecting ducts
E, mV	0	40	15
$(Na^+)_I$, meq/l.	145	145	150–450
$(Na^+)_{TF}$, meq/l.	110	25	1
$(Cl^-)_I$, meq/l.	110	110	150–450
$(Cl^-)_{TF}$, meq/l.	110	25	1
PD_{Na^+}, mV	7	87	148–177
PD_{Cl^-}, mV	0	0	118–147

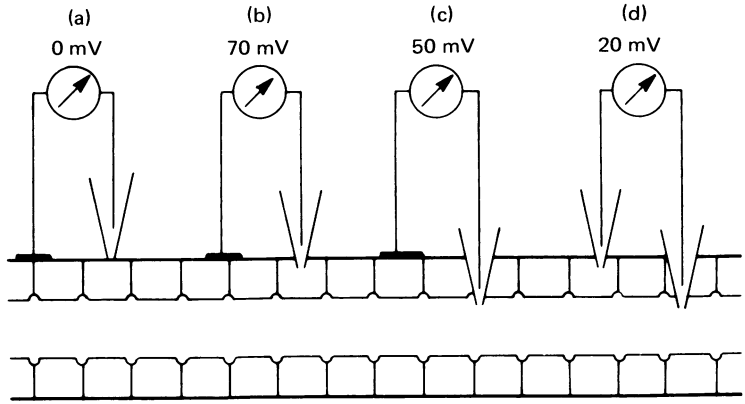


Fig. 10. A segment of the distal convoluted tubule indicating the position of electrodes when measuring transmembranal electrical potential differences (b, d) or trans-tubular potential differences (c). At (a) both electrodes are at cell surface and no difference is recorded.

interior of the cell and the lumen (Fig. 10(d)), the latter being the more positive.

The transcellular potential difference of 50 mV (lumen-negative) means that all cations that traverse the cell during reabsorption do so against an electrical gradient, whereas all anions move down an electrical gradient.

Electrolyte Transport

Approximately four-fifths of the total dissolved material in the glomerular filtrate consists of sodium salts. The reabsorption of sodium and accompanying anions (predominantly Cl^- and HCO_3^-) thus exceeds that of all other solutes.

About two-thirds of the filtered sodium is actually reabsorbed isosmotically in the proximal tubules, water being reabsorbed in the same proportion as sodium, so that the sodium concentration at the end of the proximal tubule is the same as that in Bowman's capsule.

If there are present in the tubular fluid, substances that are not readily reabsorbed but are osmotically active, (for example, dextran or mannitol), sodium and water are still reabsorbed, but a part of the water is held back

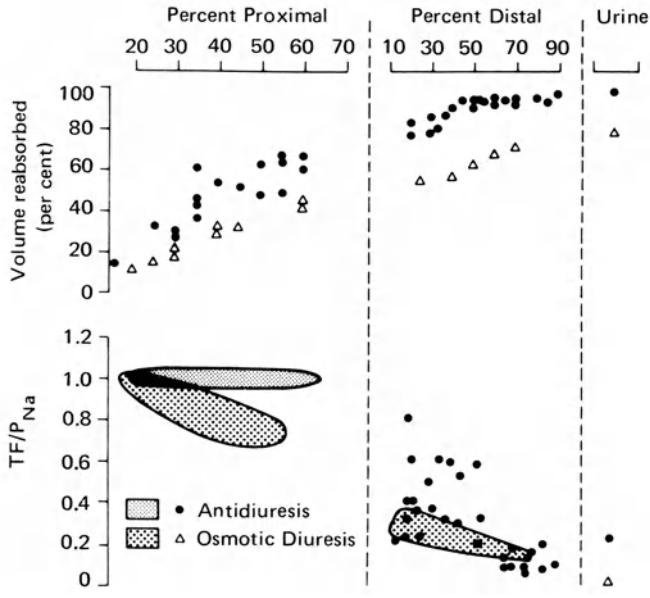


Fig. 11. Fractional reabsorption of sodium and water in several parts of the nephron during antidiuresis as compared to osmotic diuresis. $(TF/P)_{Na}$ = ratio of sodium concentrations in tubular fluid and plasma. From Giebisch and Windhager (31).

by the osmotic effect of the poorly reabsorbed substances, so that the osmotic pressure of the tubular fluid also under these conditions remains equal to that of the plasma. The consequence is a reduction in the sodium concentration in the tubular fluid, which can fall to less than 30 percent of that in the plasma (Fig. 11). Under these circumstances, even the proximal reabsorption of sodium must proceed against a concentration gradient.

The precise analysis of tubular fluid was first carried out in an amphibian creature, Necturus (the mud puppy), a type of salamander found in North American waters. This animal has the good fortune (for the physiologist) of possessing very large nephrons, which makes micropuncture experiments a great deal simpler from a technical point of view. Moreover, in cold-blooded animals transport processes, being temperature-dependent, proceed more slowly than at 37°C. Necturi live in relatively cool waters, and transport processes that take place in warm-blooded animals in a matter of seconds take many minutes at the preferred temperature of Necturi (i.e., 8 to 10°C)'

These facts make Necturus a useful subject for the split oil drop experiment (see page 16 and Fig. 6), in which a segment of proximal tubule isolated between two columns of oil is injected with a test solution. In this way a number of different test solutions may be studied in sequence, all being isosmotic but containing varying concentrations of sodium chloride. The tonicity of the solutions is kept equal to that of plasma by the addition of mannitol. Radioactively labeled inulin is included in the test solution so that, from changes in its concentration, percentage changes in the volume of water can be calculated.

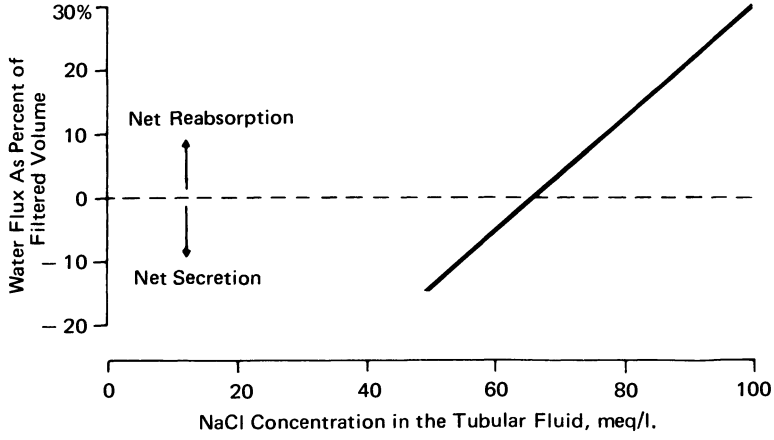


Fig. 12. Water flux in the proximal tubule of Necturus related to the intratubular concentration of sodium chloride. From Windhager et al. (111).

The result of several such experiments is shown in Fig. 12. The direction and magnitude of the trans-tubular water flux depend on the sodium concentration in the test solution. With the highest sodium concentration, i.e., 100 meq/l. (which corresponds to the normal plasma-sodium level in Necturus), 30 percent of the water was reabsorbed within 20 minutes; with a sodium concentration of 75 meq/l., scarcely 10 percent was reabsorbed; with a sodium concentration of 66 meq/l., reabsorption ceases; at lower sodium concentrations there is a net influx of water into the tubule. Since, in all cases, the solution was isosmotic, there can be no osmotic explanation for the differing water fluxes. The driving force is, in fact, the active reabsorption of sodium. This sodium can be transported against a concentration gradient with the expenditure of metabolic energy (see page 25). In the proximal tubules of Necturus the sodium pump can bring about a net transport of the ion against a concentration gradient of as much as 34 meq/l. (i.e., 100 minus 66). A concentration gradient greater than this between plasma and tubular fluid exceeds the capacity of the pump and leads to a passive influx of sodium into the tubular fluid.

An explanation of the relationship between active and passive sodium

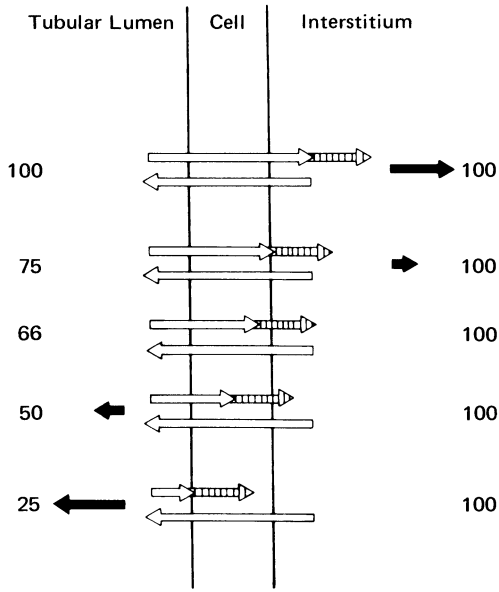


Fig. 13. Schematic summary of active and passive sodium fluxes across the wall of a proximal tubule in Necturus, demonstrating the reversal of net outward flux as the intraluminal concentration falls. The length of the arrows is proportional to the magnitude of the corresponding flux. ⇨ is active flux; ⇨ is passive flux; and ➡ is net flux.

fluxes across the tubular wall in Necturus is attempted in Fig. 13. Here the simplest case of a constant-rate and concentration-independent sodium pump is depicted.* If the sodium concentration is the same on both sides of the tubular epithelium, the passive fluxes in either direction will be identical, and any net transport of sodium is produced solely by active pumping. If the sodium concentration of fluid on the luminal side is reduced, then the passive flux from lumen to interstitium is also reduced, so that on balance there is a net flux of sodium from interstitium to lumen, even though active transport continues undiminished.

In warm-blooded animals, exactly analogous events take place (29). In these species, too, the sodium pump in the proximal tubules can maintain a concentration gradient of some 35 meq/l.; this means that with a normal plasma-sodium of 145 meq/l., the luminal concentration can fall to 110 meq/l. (in the presence of an unabsorbable but osmotically active non-electrolyte) before net outward movement of sodium comes to a standstill. The pump in the distal parts of the nephron can transport sodium against even higher concentration gradients. In the ascending limbs of the loops of Henle and in the distal convolutions, concentration gradients of at least 100 meq/l. can be established. Since in certain circumstances an almost sodium-free urine can be produced while the sodium concentration in the interstitium of the medulla can reach 450 meq/l., it follows that the sodium pumps in the collecting ducts must be able to build up and maintain very high concentration gradients.

Since about 95 percent of the dissolved substances in the glomerular filtrate that are subsequently reabsorbed in the tubules are either strong or weak electrolytes and carry electrical charges, proof of active transport requires more than the demonstration of movement against a chemical concentration gradient; it is also important to establish if an electrical potential difference must be overcome. Such an electrical potential difference arises in association with a difference in concentration of charged particles. Under equilibrium conditions (i.e., in the presence of zero net flux) the potential difference is described by the Nernst equation as follows:

$$E = \frac{R \cdot T}{z \cdot F} \times \ln\left(\frac{[C_i]}{[C_{TF}]}\right) \tag{13}$$

Where E = electrical potential
R = universal gas constant
T = absolute temperature

* Concentration—independent means a pump that transports a fixed quantity of material per unit time regardless of the concentration at which material is presented to it, provided that this is more than zero.

z = valency
F = Faraday's constant
$([C_i])$ = concentration in interstitial fluid.
$([C_{TF}])$ = concentration in tubular fluid.

If there exists a concentration ratio for which the calculated electrical potential difference is equal to the measured value, but is of opposite polarity, it can be inferred that the system is behaving passively. In the presence of active transport, appreciable discrepancies between measured and calculated potential differences are possible.

In Table 3 are assembled the data required for calculating the electrochemical potentials of the important ions Na^+ and Cl^- in the several subdivisions of the renal tubule in warm-blooded animals. Sodium is evidently reabsorbed actively along the entire length of the nephron, as opposed to electrochemical gradients, which increase in magnitude with distance from the glomeruli. With respect to the "economics" of transport processes, it is significant that the main burden of the reabsorptive process falls on the proximal tubule, where no concentration gradient exists, and that ever diminishing quantities of sodium are reabsorbed as the distal extremity of the nephron is approached.

For chloride the electrochemical potential differences in the proximal and distal convolutions are not strikingly different from zero, so that in the case of this ion, active transport would appear to be confined to the region of the collecting ducts and the loop of Henle.

Energy Metabolism of the Kidney

Transport against an electrochemical gradient requires energy that must be made available by the metabolic activity of the cells. It is generally accepted today that such an energy-requiring system is involved in sodium reabsorption and that the active transport of this ion is the primary event in the reabsorption of filtrate. Anions follow to preserve electrical neutrality and water also follows passively to maintain osmotic equilibrium.

The relation between energy utilization and active sodium transport by the dog kidney is shown in Fig. 14. By manipulation of the arterial blood supply, the GFR, and hence the sodium load presented to the tubules, was varied. Further variations in sodium reabsorption were induced by saline infusions and by partial inhibition of active transport by pharmacological agents or by oxygen deprivation. As the rate of sodium reabsorption falls, there is a proportionate reduction in oxygen consumption until, with the cessation of net reabsorption of sodium— that is, in a nonfiltering kidney— a basal level of oxygen consumption is reached. This value is of the same

order as that in other epithelial structures in the resting state. From Fig. 14 it appears that for every milliequivalent of oxygen used, 6.9 to 7.7 meq of sodium are transported. Considering that a small part of the oxygen consumed will be needed for the reabsorption and secretion of other substances, the O_2/Na may approach 1 : 8. This ratio for renal tissue exceeds that found in any other actively transporting cell. For example, in nerve the ratio of oxygen used to sodium transported is 1 : 1, while in submaxillary gland it is 1 : 2 and in pancreas it is 1 : 3.

Recently, doubts have been raised as to whether all sodium transport can be quantitatively regarded as "active." In the tubule, reflection coefficients (see page 46) for sodium chloride exist that are significantly less than 1 (108). This suggests a certain passive permeability of the tubular wall to sodium chloride, so a portion of it could be carried passively through the tubular wall by what is known as "solvent drag," associated with the transmural flux of water (see page 46). If, then, an active transport of

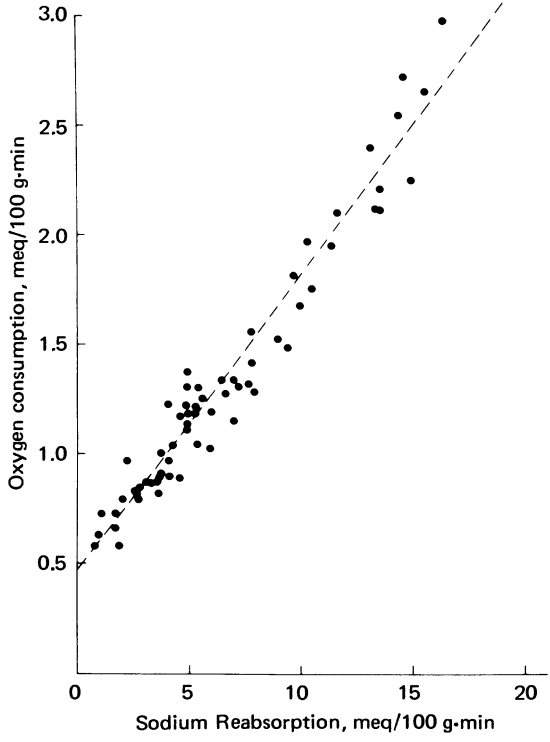


Fig. 14. Relationship between oxygen consumption and sodium reabsorption in the dog kidney. Sodium reabsorption was varied by changing the filtered load and by partial blockage of the transport system with drugs. Modified from Deetjen and Kramer. (18).

sodium and an accompanying passive movement of chloride creates an osmotically driven flux of water, some additional quantities of Na^+ and Cl^- would be drawn out of the tubular lumen passively. When allowance is made for this solvent drag effect, the ratio of sodium actively transported to oxygen used is somewhat reduced.

Ultrastructure of the Nephron

In conformity with their large energy turnover, the cells of the proximal convoluted tubule exhibit a high degree of morphological differentiation. Their dense population of unusually long mitochondria ("the powerhouses" of the cell) is a prominent feature in electron micrographs (Fig. 15). Also, in regard to the number and quantity of their enzymes, these cells surpass those in all other parts of the nephron (100). On the luminal side, the presence of the so-called brush border (consisting of bristle-like projections) increases the reabsorptive surface in contact with tubular fluid some 60-fold. The same purpose seems to be served on the basal aspect of the cells by infoldings of the cell membrane which in places form a labyrinth of canaliculi penetrating deeply into the cell substance. A basement membrane, similar in its electron-microscopic appearance to that present in the glomeruli, forms the boundary between the tubular epithelium and the endothelium of the peritubular capillaries. Substances that are filterable at the glomerulus can also pass freely through this basement membrane. It seems that all substances that pass through the tubular cells into the interstitium or from the interstitium into the cells are routed through the canaliculi of the basal labyrinth. The walls of the canaliculi have a structure similar to the so-called plasma membrane found in all intracellular organelles such as mitochondria, lysosomes, centrioles, ribosomes, and Golgi apparatus. Under the electron microscope (Fig. 16(a)) a plasma membrane when fixed in osmic acid is seen to comprise three layers. Figure 16(b) is a schematic representation of the molecular structure of the plasma membrane. The translucent middle layer corresponds to a double row of adjacent phospholipid molecules of which the negatively charged and hydrophilic phosphate groups are outwardly directed. The closely packed "tails" of the phospholipid molecules in the interior of the membrane are nonpolar and hydrophobic. The central lipid layer is covered on both sides by a single or multilayered sheet of protein. The electron-dense osmic acid is not lipid-soluble, and can thus associate only with the protein layer and the hydrophilic groups in the phospholipids.

The fact that water and lipid-insoluble substances (such as strong and weak electrolytes and glucose) can traverse plasma membranes under certain conditions leads to the postulation of membrane pores which must

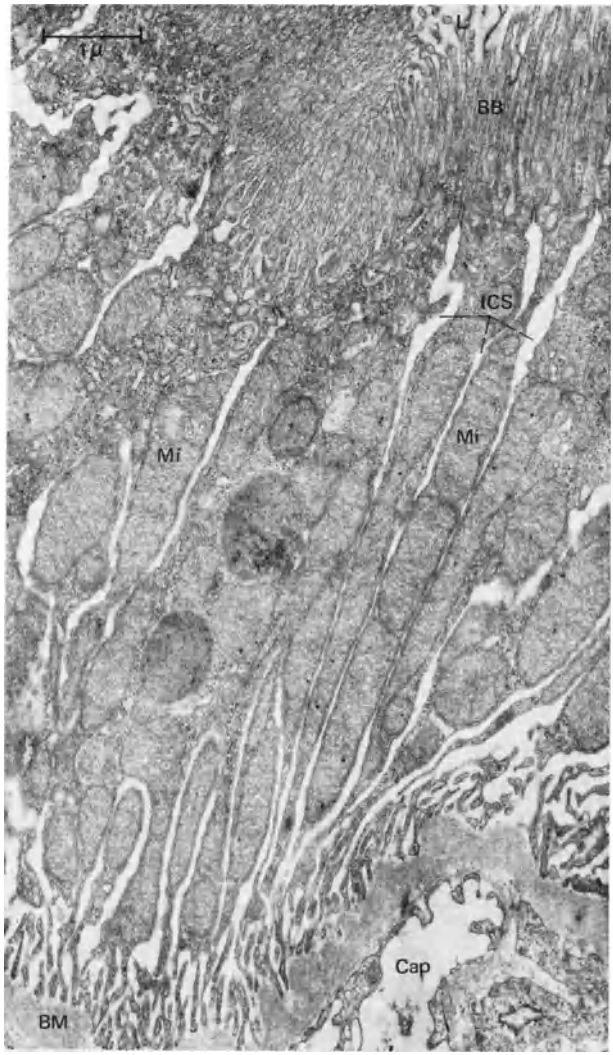


Fig. 15. Electron micrograph of a section of proximal convoluted tubule with a number of epithelial cells cut across. The brush border (BB) can be seen projecting into the collapsed lumen (L). In the interior of the cell numerous elongated mitochondria (Mi) are lying between deep infoldings of the basal membrane of the cell. ICS = intercellular cleft; BM = peritubular basement membrane; Cap = peritubular capillary. Magnification: 12,500:1. Electron micrograph taken by W. Thoenes.

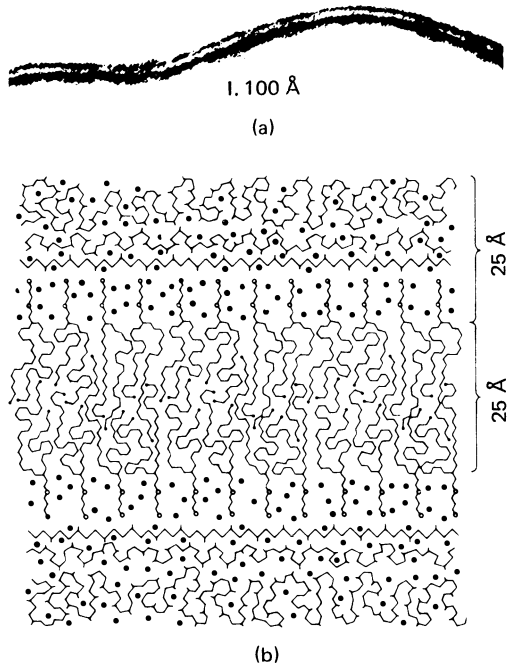


Fig. 16. (a) Electron microscopic picture of a plasma membrane after fixation with osmic acid. Magnification is 500,000:1. (b) Schematic representation of the proposed structure of the plasma membrane showing the central bimolecular layer of phospholipids with the polar groups directed outward and bordered with overlying layers of protein on each surface. OsO_4 stains only hydrophilic material, which explains the appearance in (a). From Robertis et al. (20).

have a diameter of about 3Å. This is below the resolution of electron microscopy and no such structures have actually been demonstrated. It is possible that the pores in question may not be static pores, fixed in position, but rather transient breaches in the membranous structure. The plasma membrane appears, in fact, to be the principal impediment to substances transported across tubular cells.

In the distal tubule—as in all segments of the nephron apart from the proximal tubules—a brush border is lacking. The mitochondria are less densely packed and the basal labyrinth is less copiously branched. Enzymes are present in equal variety but in lower concentrations than in the proximal cells. The same applies to the thick ascending segment of Henle's loop, while the thinner segment reveals very little of a recognizable ultrastructure. Here, the flat epithelial cells contain only a few small mitochondria and are scarcely distinguishable from those forming the endothelium of the

neighbouring vasa recta. In agreement with the morphological picture, there is a striking poverty of enzymes, especially those involved in oxidative metabolism. The cells of the collecting ducts are relatively large and possess numerous basal invaginations, but are otherwise morphologically and histochemically as poorly differentiated as the cells of the thin portion of Henle's loop. In all segments of the nephron the enzymes concerned with anaerobic energy production are found in relatively high concentrations.

Trans-tubular Water Flux

The magnitude of trans-tubular water flux, particularly in the proximal tubules, poses several problems. With special micropuncture techniques it has been possible to measure the water permeability (i.e., the hydraulic conductivity) of the proximal tubular wall (103). This has a value of 17.4 $\times 10^{-8}$ ml/cm^2/sec/cm H_2O and is about 20 times less than that of the glomerular membrane (see page 8). In order to draw through the proximal tubular cells the 60 percent of glomerular filtrate that is normally reabsorbed, an osmotic gradient between the luminal fluid and the interstitium of 20 mosm/l. would be required. However, the tubular fluid is actually isotonic with the interstitial fluid, so there is in fact no osmotic gradient between them.

As a description of the phenomenon of iso-osmotic water transport, the model of Curran and MacIntosh (Fig. 17, ref. 14) has attracted considerable attention. It consists of three rigid compartments: A, B, and C. Of these, A and B are separated from each other by a cellophane membrane with wire mesh support, while a sheet of sintered glass is interposed between B and C. Osmotically driven movement can take place only between A and B, and not between B and C, because the pores are too large. Raising the osmotic pressure in B leads to an osmotic flow of water from A to B. This creates a hydrostatic pressure in B that leads to the passage of water from B to C independent of the osmotic pressure in C. This mechanism (81, 103) could operate in the tubular cell in the following way: Utilizing the metabolic energy provided by the mitochondria, active transport of sodium could take place in the intercellular channels (corresponding to compartment B in the model), where the osmotic pressure would become slightly raised. Water would follow and cause an increase in the hydrostatic pressure in the intercellular channels, with a resulting passage of water in the direction of the interstitium. In this way water flow across the epithelium could be a predominantly hydrodynamic process, although initiated by a local osmotic gradient in the intercellular channels.

It should be mentioned that water movement across an epithelial sheet occurs also in a number of other organs without any osmotic gradient

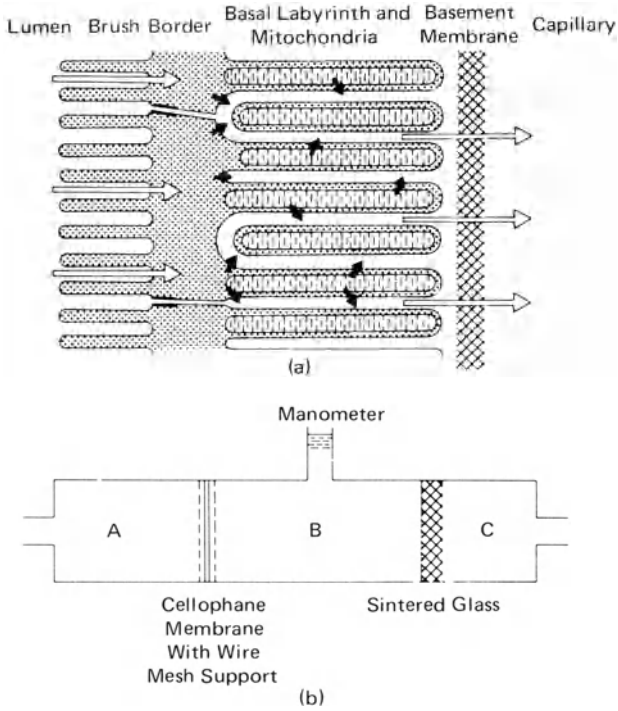


Fig. 17. (a) Diagram of a proximal tubular cell. Open arrows indicate a passive flux of NaCl and water, while solid arrows indicate active transport of NaCl. (b) The model proposed by Curran and MacIntosh (14) to explain the mechanism of isotonic fluid transport across a cell.

being demonstrable. This happens in the stomach, small intestine, gall bladder, liver, pancreas, eye, and the choroid plexus, which produces the cerebrospinal fluid. In these situations the cell structure is similar to that found in the renal tubules, with many mitochondia present and the characteristic intercellular channels (68).

Peritubular Factors Affecting Reabsorption

To complete the process of reabsorption, tubular fluid that has moved from lumen to interstitium must return to the blood via the peritubular capillaries. This so-called end-stage transport (Fig. 17) is a passive process driven by the oncotic pressure difference between interstitial fluid and plasma. As a result of the high resistance of efferent arterioles, the fall in pressure beyond the glomeruli is so large that in the peritubular capillaries the same low hydrostatic pressure (about 15 mm Hg) results as in the capsular space of the glomerulus and in the proximal tubule. However,

because of the abstraction from glomerular blood of the protein-free filtrate, the colloidal osmotic pressure of blood passing from the glomerular tuft to the peritubular capillaries is raised, increasing the oncotic gradient (see page 9), which causes water to flow from interstitium into capillaries (56).

It must be pointed out that these peritubular factors exert only a modifying or regulating influence on the total process of reabsorption and in no sense provide its driving force. Because of the low water permeability of the proximal tubular wall, the colloidal osmotic pressure of the capillary blood alone could account for only 1 percent of the observed amount of filtrate reabsorbed. Also a reversal of the oncotic gradient induced by injecting protein-containing solutions into the tubular lumen produces no appreciable reduction in the reabsorptive capacity of the tubule.

The peritubular factors that affect reabsorption also afford a possible explanation of the observation made some time ago that in the face of spontaneous or experimentally induced variations in the GFR, fractional (or percentage) reabsorption in the proximal tubules remains unchanged. This phenomenon has been called *glomerular-tubular balance*, and means that in response to changes in GFR, a constant percentage of the filtered load of sodium and water are reabsorbed. The absolute amounts reabsorbed will vary with the filtration rate. Since the final step of the reabsorptive process depends on the oncotic pressure of the plasma in the peritubular capillaries and since this in turn is determined by the glomerular filtration rate, the matching of proximal reabsorption to filtration rate could be ascribed to a peritular controlling mechanism.

The peritubular factors regulating reabsorption in the proximal tubules also seem to have influence on the control of sodium excretion. This becomes clear when the balance between the individual steps in the reabsorptive process is disturbed by the infusion of sodium chloride. When such an infusion is given, the plasma proteins are diluted and the colloidal osmotic pressure of the plasma is reduced, leading to an increase in GFR. However, since renal blood flow is increased even more, the net result is a reduction in the filtration fraction, and hence in the colloidal osmotic pressure in the peritubular capillaries. Since this pressure affects the final step in the reabsorptive process, a reduction in it will result in a depression of sodium reabsorption. The consequence is an increase in the delivery of sodium-containing tubular fluid to the distal nephron. Under these conditions also aldosterone activity is reduced (see page 126), and so the transport capacity of the distal nephron is insufficient to cope with the increased sodium load. Hence, more of the sodium that reaches the distal tubule escapes reabsorption and appears in the final urine. This relationship between the sodium "rejected" (i.e., the percentage of the filtered load excreted in the urine) and the peritubular oncotic pressure is diagramed in Fig. 18.

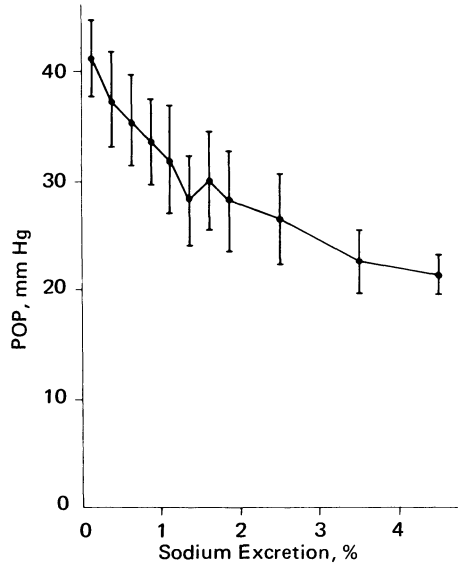


Fig. 18. The relationship between oncotic pressure in the peritubular capillaries (POP) and sodium excretion (as a percentage of the filtered load) in experiments on dogs. Unpublished results of Keck; see also Kramer, Boylan, and Keck (47).

Effect of Aldosterone on the Nephron

Whereas less than 1.0 percent of filtered sodium is normally excreted, the proportion may rise in the presence of adrenal insufficiency to between 2 and 3 percent of the filtered load; this situation can be corrected by the administration of adrenal extracts. The mineralocorticoid aldosterone, first discovered in 1953, is the main hormone involved. Apparently aldosterone can promote sodium transport not only in the kidney but in all other epithelial structures that exhibit net transport of the ion. This has been observed in the intestine, sweat glands, and salivary glands, as well as in frog skin and toad bladder, which are the favored preparations for *in vitro* studies.

The reduction in sodium reabsorption seen in the presence of adrenal insufficiency or after adrenalectomy depends largely on a diminution in reabsorption by the distal segment of the nephron. Whereas normally the sodium concentration in the distal convoluted tubule is about one-quarter of that in plasma (see page 25), this may rise in the adrenalectomized rat to one-half or more. The epithelium of the distal tubule is, therefore, unable, after extirpation of the adrenal glands, to maintain as great a concentration gradient for sodium as it can when the adrenals are intact (40).

However, the effect of aldosterone is not confined to the distal nephron, for in micropuncture experiments using the split oil drop technique (see page 16) it was found that the time required for sodium reabsorption was doubled in the proximal convolutions of adrenalectomized rats. If aldosterone is administered, this depression of the local reabsorptive capacity can be reversed in the proximal as well as in the distal convoluted tubule. The loss of aldosterone action in the proximal tubule seems, nevertheless, to have no effect on glomerulo-tubular balance, since the percentage of the filtered load of sodium that is reabsorbed remains unchanged (40). The most plausible explanation for this "compensatory" mechanism is that the passive reflux of sodium into the tubular lumen is depressed in the absence of aldosterone to the same degree as is the active transport process concerned in reabsorption. There is, in fact, experimental evidence for both these effects of aldosterone in frog skin and toad bladder (22, 55).

It should be noted that not only mineralocorticoids but also glucorticoids (e.g., cortisone) have a direct action on tubular function. Following adrenalectomy, the excretion of a water load is delayed. This delay depends on an enhancement of the water permeability of the distal tubule and can be returned to normal by the administration of glucocorticoids. This alteration of water permeability is a direct effect of the gluco-corticoids and cannot be brought about either through an influence on antidiuretic hormone (ADH) activity or through changes in sodium or potassium reabsorption (90).

Calcium Reabsorption in the Kidney

Ca^{++} ions are handled by the kidney in very much the same way as Na^{+} ions. If correction is made for the fact that only about one-half of the calcium in plasma is present in the form of free ions, it is found from clearance (107) and from micropuncture studies that Ca^{++} and Na^{+} are reabsorbed by the tubule in similar proportions to those found in the glomerular filtrate. Throughout the length of the proximal tubule the concentration ratio between tubular fluid and plasma (TF/P) remains at unity for Ca^{++}, exactly as it does for Na^{+}.

Tubular Transport of K^{+} in the Kidney

Normally much less potassium appears in the bladder urine than is filtered at the glomeruli. But when the intake of potassium is very high, the amount excreted may exceed the amount filtered. The tubule must therefore be capable of both net reabsorption and net secretion of potassium.

This finding was clarified by direct observations on single nephrons

using micropuncture techniques (for review see ref. 60). These revealed that the greater part of potassium filtered was reabsorbed in the proximal tubule and that at the end of this segment only about 20 percent of the filtered potassium remained (Fig. 19). This proximal reabsorption of potassium is independent of both dietary intake and the potassium balance of the animal.

All variations in potassium excretion are effected in the distal parts of the nephron. In the case of a potassium-deficient animal, the potassium that has escaped proximal reabsorption is reabsorbed from the distal tubular fluid so that only about 1 percent is finally excreted. In the presence of potassium excess in the organism, potassium is secreted into the tubular fluid by the distal segments of the nephron (3).

In the experiments shown in Fig. 19 a maximal excretion of potassium was induced by (1) supplementing a low-sodium diet with potassium, (2) infusing poorly reabsorbed anions, (3) and inhibiting H^+ ion secretion. All

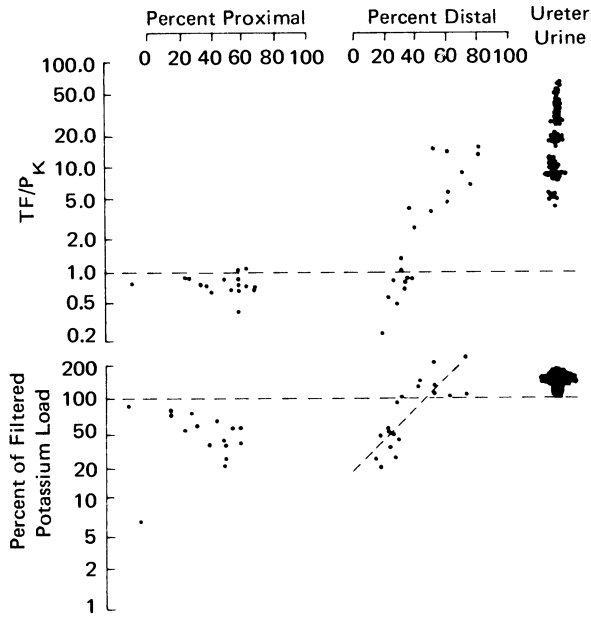


Fig. 19. Potassium transport in the proximal and distal segments of the nephron in rats. Maximum potassium excretion was induced by feeding a high-potassium, low-sodium diet, together with the infusion, during micropuncture, of sodium sulfate, potassium chloride, and a carbonic-anhydrase inhibitor. A considerable increase in potassium concentration is seen in the distal tubule (above) when the amount of potassium found exceeds that filtered, although about 70 per cent of the filtered load has been reabsorbed proximally (below). Modified from Malnic et al. (60).

these measures promote potassium secretion, so that as much as 160 percent of the potassium filtered may appear in the bladder urine.

The mechanism of distal potassium secretion is not yet completely elucidated. The early concept of a carrier-mediated exchange pump for Na^+ and K^+ has not been confirmed experimentally. At least in the rat neither the sodium concentration in tubular fluid nor the quantity of sodium reabsorbed distally appears to be a limiting factor to potassium secretion (60).

It is not necessary on the basis of present knowledge to postulate an active transport mechanism for distal potassium secretion. The transcellular electrical potential difference in the distal convolutions and collecting ducts (see page 21), where the luminal surface is negative to the peritubular surface, would favor an accumulation of potassium in tubular fluid. Even when maximal secretion is taking place, the concentrations of potassium found in distal tubular fluid are always less than the theoretical limit as a result of purely passive equilibration (see page 25). The increase in K^+ secretion that occurs with sodium deficiency in the presence of poorly reabsorbed anions in the tubular fluid (Fig. 19) can be similarly explained, since under these conditions the transcellular potential difference is almost doubled.

This increase in transcellular potential probably arises because sodium deprivation leads to an increased output of aldosterone (see page 125), which in turn stimulates the sodium pump in the distal tubule. On the other hand, the presence of poorly reabsorbed anions in the tubular fluid prevents an increase in sodium reabsorption, and a situation arises that favors a distortion of charge in the membrane and so a rise in the transmembrane potential.

Reabsorption of Glucose in the Kidney

Through the development of specific and highly sensitive enzymatic assays for glucose combined with modern micropuncture techniques, our knowledge of the renal reabsorption of glucose has been greatly extended in recent years, and many established notions have required revision.

Until recently the urine of normal people was thought to be sugar-free. But with modern analytical methods it is possible to detect traces of glucose in the urine, even when the plasma glucose level is within normal limits, and to demonstrate a hyperbolic relationship between glucose concentration and rate of urine flow (Fig. 20). From the hyperbolic function $y = a/x$ it may be inferred that the product of flow rate and glucose concentration must remain constant. This means that in the normal range of concentrations of glucose in the plasma, individuals with healthy kidneys excrete a constant quantity of glucose per unit time. This amounts to about 400 μM/

day or 0.3 μM/min (79). Since, with an average GFR of 120 ml/min and an average plasma glucose concentration of 6 mM/l., some 720 μM of glucose will be filtered each minute; the amount excreted is very small compared to the amount reabsorbed. When plasma glucose rises to abnormally high levels, even a healthy kidney fails to reabsorb the filtered glucose, and glucose then appears in large quantities in the urine as in diabetes mellitus.

The proximal tubule was recognized as early as 1941 by Walker and his associates (106) as the site of glucose reabsorption. These authors had punctured with micropipettes superficially situated renal tubules in anaesthetized rats and had analyzed their fluid. More recent micropuncture experiments, combined with sensitive enzymatic methods for the determination of glucose, confirm that the major part of glucose reabsorption takes place in the initial portion of the proximal tubule. The glucose concentration falls off steeply in the first third of the proximal convoluted tubule and then levels off at a low value (Fig. 21, ref. 80). On the other hand, it turns out from experiments in which different portions of the proximal tubule were artificially perfused that the absorptive capacity is essentially the same along the whole convoluted portion of the tubule (16). Clearly a certain limiting concentration can be attained in the tubular fluid, and as the

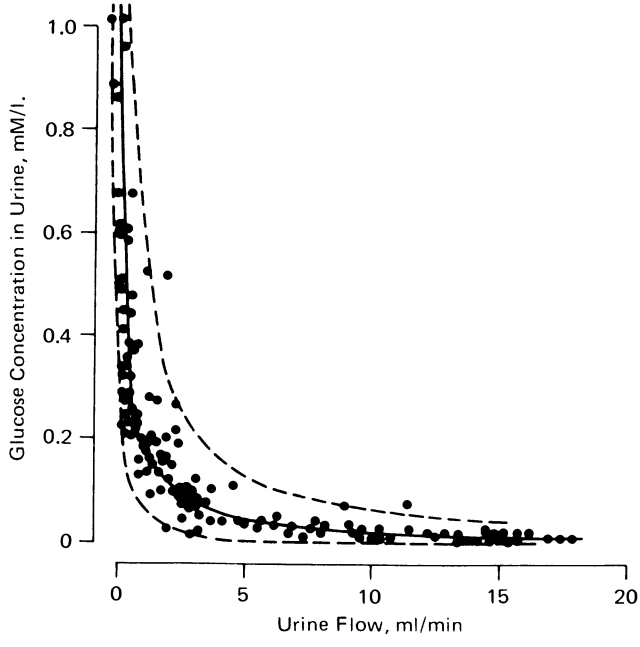


Fig. 20. Relationship between the urinary concentration of glucose and urine flow rate. From Renschler (79).

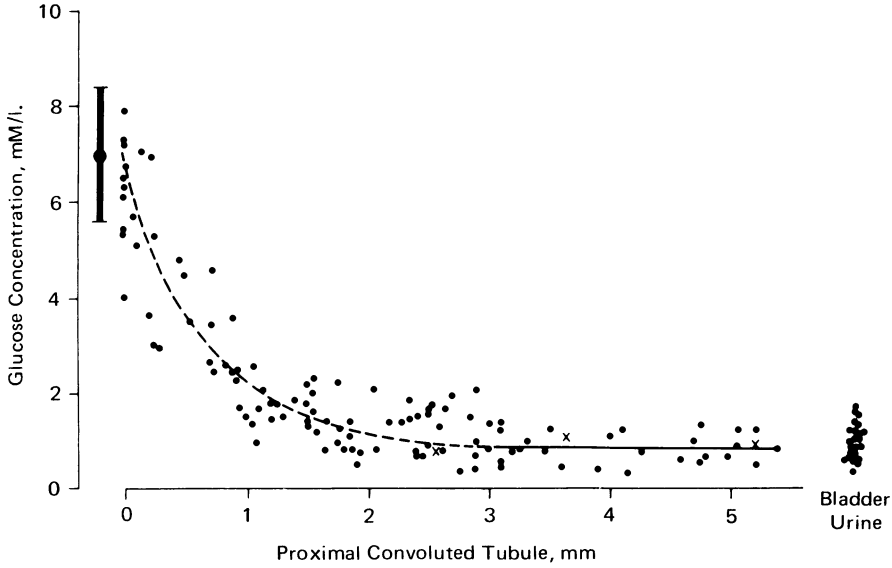


Fig. 21. The fall in glucose concentration along the proximal tubule, as observed in micropuncture experiments in rats. The range of values for normal plasma glucose is indicated by the vertical bar. From Rohde and Deetjen (80).

plasma concentration of glucose rises, successive segments of the tubule are fully committed to glucose reabsorption. If the plasma concentration rises so high that the total absorptive capacity no longer suffices to reduce the tubular concentration to the limiting value, correspondingly greater amounts of glucose are excreted.

The distal tubules are not credited with any capacity to reabsorb glucose, but in fact, no direct evidence is available. It is known, however, from rat experiments that, at normal plasma concentrations, glucose appears in the bladder urine in—at most—double the concentration found at the end of the proximal convolutions. Since at the same time substances such as inulin, which are not reabsorbed, are concentrated at least 100-fold between the end of the proximal tubule and the renal pelvis, one must conclude that glucose is also removed by segments of the nephron beyond the proximal tubule. It is not yet decided to what extent a genuine transtubular transport for glucose is involved in this distal reabsorption or if the glucose is utilized for metabolic processes in the renal medulla. Actually it is known that, in contrast to the cortex, the medulla has a relatively high rate of glucose consumption (100).

Hitherto it has been assumed (83, 86) that proximal tubular cells have a limited capacity to reabsorb glucose, which is characteristic of the species and the individual and is not influenced by other factors affecting renal

function. The magnitude of the maximal possible rate of glucose reabsorption, the so-called transport maximum (Tm) for glucose, would then depend solely on the mass of reabsorbing cells present. This concept, however (on which a clinical test is also based), now requires revision. Experiments on rats have in fact demonstrated that in the presence of an excessive load of glucose, which should call for maximal reabsorption, the transport rate actually varies with the rate of salt and water reabsorption (80, 105). In view of this relationship, it would seem that there must exist a kind of glomerulo-tubular balance (see page 32) with respect to glucose reabsorption, the mechanism of which, as is the case with salt and water reabsorption, is still somewhat obscure.

The response of the kidney to increasing glucose loads is best described by a so-called titration curve. If, while the GFR remains constant, the glucose load presented to the tubules is progressively increased by raising plasma glucose concentration, a point is reached at which the reabsorptive mechanism is fully saturated (threshold for maximal excretion). Any further rise in plasma concentration above this threshold results in a proportionate increase in the quantity of glucose excreted. However, if a similar variation in the glucose load is produced by varying the GFR, this is accompanied by a change in the apparent transport maximum, because then an increased reabsorption of water and salt is accompanied by an increased reabsorption of glucose.

A typical example of a glucose titration curve obtained from a human subject is shown in Fig. 22. It is understandable in view of what has been said that the threshold for maximal excretion is reproducible in both a single individual and large groups of subjects. The so-called mean threshold, (extrapolated from the straight portion of the curve) and also the slope of the straight portion of the curve, are susceptible to variation. Both of these are dependent on the rate of proximal fluid reabsorption and fluctuate with GFR. Note that GFR can, in fact, be calculated from the slope of the graph.

The curved portion of the graph, commonly called the splay, varies from species to species and is less marked when the threshold for maximal excretion is low and when the proximal tubules are of uniform length.

Not a great deal is know about the mechanism of glucose reabsorption. There is, however, little doubt that the process involved is one of active transport, because the almost complete reabsorption that occurs must involve movement against a concentration gradient. Experiments using isotopic labeling have shown that glucose can move from tubular lumen into peritubular capillaries within a few seconds and without disruption of its carbon chain (10). Moreover, agencies that interfere with energy production from oxidative metabolism, such as oxygen lack, reduced temperature, cyanide poisoning, or changes in pH—all inhibit glucose trans-

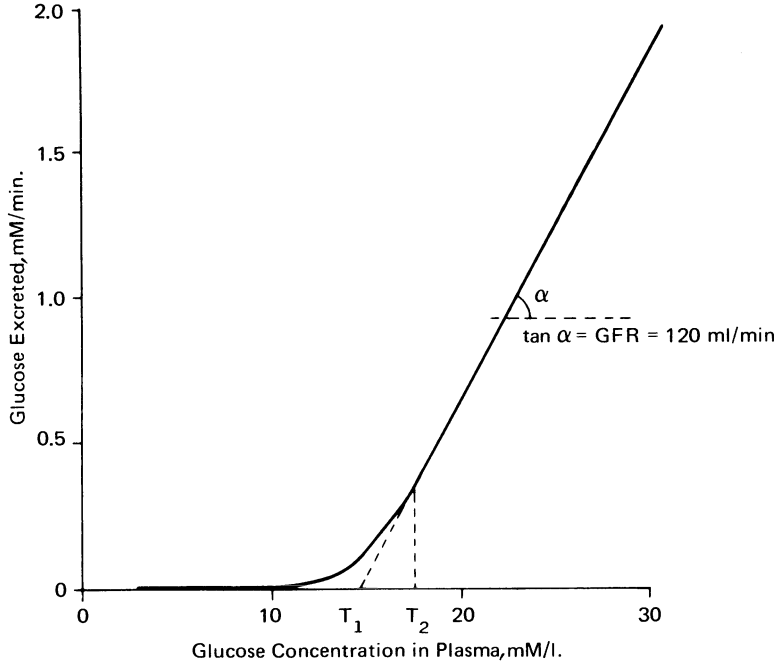


Fig. 22. Titration curve for glucose. T_1 = mean excretion threshold; T_2 = maximum excretion threshold.

port. On the other hand, dinitrophenol, which uncouples oxidative phosphorylation and is a powerful inhibitor of sodium transport, has no effect on glucose transport. Phlorizin, a gluconide obtainable from the root of fruit trees, blocks the transport of glucose as well as of certain other monosaccharides, not only in the kidney but also in the intestine and the red cells. The exact point of action of this substance is not entirely clear. It is known, however, that glucose can move passively through the wall of the proximal tubule only with difficulty because it is not lipid-soluble and therefore cannot diffuse readily through the plasma membrane that surrounds each tubular cell. Thus, aglomerular fish have only traces of glucose in their tubular fluid, even when the plasma concentration is raised several-fold above normal by infusion of glucose and the reabsorptive ability of the tubular cells is inhibited by phlorizin (61).

Phosphate Reabsorption in the Kidney

There are certain similarities between the tubular reabsorptive mechanisms for glucose and phosphate. Inorganic phosphate is apparently re-

absorbed solely in the proximal tubule (91) and as long as the GFR remains constant, a titration curve for phosphate similar to the one in Fig. 22 can be constructed (72). In glucose the threshold concentration is so high that under normal circumstances it is not exceeded, so that the kidney normally has no regulatory influence on the plasma concentration of glucose. The threshold concentration for phosphate, on the other hand, lies close to the normal plasma level, and hence the kidney must be involved in the control of the phosphate concentration in the plasma. In exercising this function it is subject to extra-renal influences. Thus, cortisone, an adrenocortical steroid, lowers the phosphate threshold, leading to an increase in phosphate excretion. Herein lies a cause of the calcium and phosphate loss found in Cushing's syndrome, which is characterized by adrenocortical hypersecretion. The result is a demineralization of the skeleton with a tendency to the development of spontaneous fractures. The hormone of the parathyroid glands has a similar effect in that it mobilizes calcium and phosphate from the bones and also lowers the renal threshold for phosphate, leading to phosphaturia, i.e., an excessive excretion of phosphate in the urine.

Sulfate Reabsorption in the Kidney

Animal protein contains about 1 percent of sulfur in the form of sulfur-containing amino acids, the breakdown of which leads to the formation of sulfuric acid, which must be buffered. The kidney then eliminates from the body the excess hydrogen ions while reconstituting the bicarbonate that has been used in the buffering process (see page 47). The sulfate itself is excreted, and the quantity involved is about 30 mM per day on a daily intake of 100 grams of protein. The renal handling of sulfate, as in the case of phosphate, is characterized by a low threshold, the plasma concentration being maintained at about 0.8 mM/l. (58). Thus, with a GFR of 170 liters a day, only $170 \times 0.8 = 136$ mM of sulfate will be reabsorbed each day. Any transitory elevations in the plasma concentration due to protein catabolism that exceed the threshold will lead to sulfate being excreted in amounts proportional to the amount filtered.

Reabsorption of Amino Acids in the Kidney

There is at present very little reliable information regarding the tubular transport of amino acids. However, it can be stated with confidence that normally 98 percent of filtered amino acids are reabsorbed and that this takes place predominantly in the proximal tubules. When the plasma concentration of amino acids rises, so does their rate of excretion. However, a

typical titration curve resembling the one for glucose has, so far, been described only for arginine (28, 70). There are no corresponding observations for the other amino acids.

In recent years a great number of pathological amino-acidurias (i.e., conditions in which there is an excessive urinary excretion of amino acids) have been recognized. Some of these conditions arise from a genetic defect in proximal tubular cells, which are then not able to transport either a particular amino acid or a group of chemically related amino acids. Alongside these so-called primary amino acidurias there exist a number of secondary forms, which arise as sequelae of various diseases—mostly of obscure causation (6).

Uric Acid Transport

In mammals urinary nitrogen is mainly in the form of urea (see page 42). Only about 5 percent appears as uric acid, which arises from the metabolism of nucleic acids, but the excretion of uric acid is of practical importance because of the part it plays in certain disease processes, e.g., gout and renal stone formation.

Uric acid is freely filterable, but only little more than 10 percent of the filtered load appears in the urine (3). The proximal tubule and Henle's loop have been identified as major sites of reabsorption. Clearance studies have suggested that some uric acid might also be secreted (1), and micropuncture experiments have shown that the proximal tubule is the site of secretion; it seems that uric acid can make use of the transport system for organic acids which is located there (see page 58). If the experimental conditions are chosen so that the reabsorption of fluid in the proximal tubules is impaired, then the rate of uric acid secretion may exceed its simultaneous reabsorption rate by two or three times. However, under conditions of free flow, net secretion is replaced by net reabsorption, since, as a result of reduction in the volume of tubular fluid, a steep concentration gradient for uric acid is built up so that unidirectional diffusion from lumen to interstitial fluid outweighs secretory transport in the opposite direction (36).

Reabsorption of Protein in the Kidney

In normal man the amount of protein excreted in the urine amounts to no more than 100 milligrams a day, but there is good reason to believe that this represents only a fraction of the protein filtered. Undoubtedly the glomerular basement membrane largely prevents leakage of protein, and in micropuncture experiments on rats and dogs, protein concentrations of 10

milligrams or less per 100 milliliters have been found in proximal tubular fluid, as compared with concentrations in plasma of around 6 grams per 100 milliliters. However, since in the course of a day the filtered volume in man is around 170 liters, the amount of protein filtered could amount to between 4 and 17 grams, of which up to 99 percent must be reabsorbed.

As regards the mechanism of protein reabsorption, it is known from electron-microscopic observations that protein markers can be taken up by pinocytosis from the lumen into the cells of proximal tubules (62). During the migration of the pinocytotic vesicles toward the basal pole of the cell, proteolytic enzymes enter them from the lysosomes. The volume of these vesicles decreases progressively, presumably because proteins are broken down by enzymatic action into fragments of sufficiently small molecular size to pass into the cytoplasm (82).

Transport of Urea

Urea is the principal end product of protein catabolism in most vertebrates (see page 91 for exceptions). It is apparently not toxic to the organism, and an accumulation of it in the extracellular fluid in concentrations many times greater than normal is apparently not harmful. When in clinical cases of renal insufficiency the condition of *uremia* (i.e., excess urea in the blood) develops, symptoms are not due to urea but to the accompanying disturbances in electrolyte and acid-base balance. However, since urea is easily measured, its concentration is used as a convenient indicator of the gravity of the other disturbances.

Even in persons with healthy kidneys the blood urea level is not very constant. It can vary about a mean value of 4.5 mM/l. (27 milligrams per 100 milliliters), depending simply on the protein content of the diet. Since the urea clearance in man ranges from 50 to 60 percent of the inulin clearance, it follows that between 40 and 50 percent of filtered urea must be reabsorbed during passage of the glomerular filtrate along the tubules. If GFR falls, urea clearance also falls, and urea will be retained in the plasma. The relationship between protein intake, GFR, and plasma urea concentration is illustrated in Fig. 23: an average blood urea of 27 mg/100 ml correspond to a daily protein intake of 1.0 g/kg body weight. The same protein intake in the presence of a 40 percent reduction in the GFR leads to a blood urea of 44 mg/100 ml, whereas if the GFR is reduced by 80 percent, the blood urea will rise to 128 mg/100 ml (see the dashed line in Fig. 23). Conversely, blood urea will remain constant if protein intake is reduced along with a falling GFR (see the dotted line in Fig. 23).

The clearance of urea is also influenced by the rate of urine flow. As can be seen from Fig. 24, the urea clearance exhibits a slight but significant

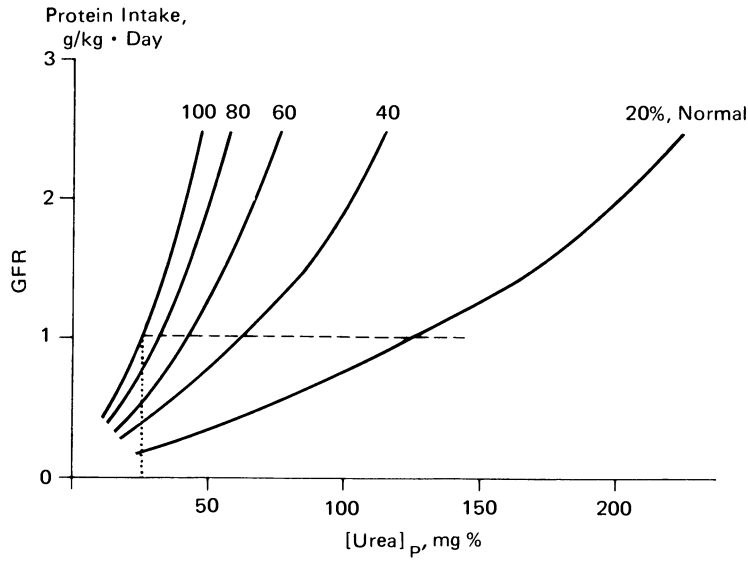


Fig. 23. The dependence of plasma-urea concentration on protein intake at different glomerular filtration rates. $[Urea]_P$ = plasma urea concentration. Modified from Bull (7).

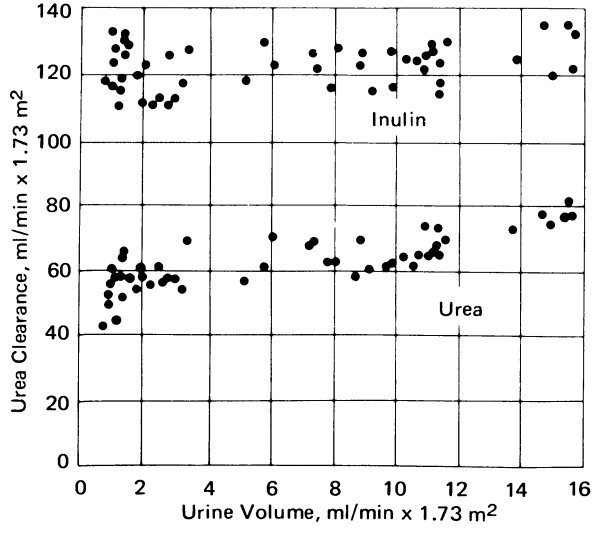


Fig. 24. Comparison of urea clearance with inulin clearance (i.e., GFR) at increasing rates of urine flow. Data from man. From Chasis and Smith (8a).

rise as urinary flow rate increases, although inulin clearance remains unchanged. This dependence of urea clearance on urinary volume suggests that the reabsorption of urea is a passive rather than an active transport process.

Two main mechanisms concerned with the passive reabsorption of non-electrolytes merit some discussion: these are *back diffusion* and *solvent drag*. The net flux ($\dot{n}/q$) of a substance that diffuses in unit time across a boundary of thickness x and area q is dependent upon the concentration difference across the boundary. This relationship is expressed in Fick's Law, which can be written as follows:

$$\dot{n} = \frac{dn}{dt} = -qD\frac{dc}{dx} \text{ (moles/sec)} \tag{14}$$

In considering diffusion across a biological membrane such as the tubular wall, it is advantageous to combine the terms D and Δx into P, the so-called permeability coefficient. $P = D/\Delta x$ will have the dimensions of cm/sec, and this parameter can be measured more readily than the separate values of D and Δx. The quantity (Φ) of substance undergoing back diffusion over unit area is then given by

$$\Phi = P \cdot \Delta c \text{ (moles/sec cm}^2\text{)} \tag{15}$$

In the case of back diffusion out of the renal tubule, ΔC is represented by the difference in concentration of the substance between tubular fluid and plasma water ($C_{TF} - C_{PL}$).

By solvent drag is meant the carriage of dissolved material by a stream of solvent. The solvent-drag effect in the renal tubule is due to the transtubular net flux of water (Φ H_2O in ml/sec cm^2), and is affected by the relative permeability of the tubular wall to the solute in question as compared with its permeability to water. As a measure of this relative permeability, one may use the so-called reflection coefficient σ. This has a value of unity when a membrane is completely impermeable to a particular substance, in which case all of that substance dissolved in the moving water would be "reflected" at the membrane. If no such reflection occurs, then the substances can pass freely through the membrane, along with the water in which it is dissolved; the reflection coefficient then becomes zero and solvent drag operates maximally. Insofar as the system approaches equilibrium, i.e., when the concentrations in the two phases separated by the membrane are not widely different, the solvent-drag effect can be formulated mathematically (44) by inserting the mean concentration in the two phases into the equation:

$$\Phi = \left[1 - \sigma\,\Phi H_2O\left(\frac{C_{TF} + C_{PL}}{2}\right)\right] \tag{16}$$

With micropuncture techniques (see page 14) it has been possible to study in rats the efflux of urea from the tubule under conditions when trans-tubular water flux is zero. From these studies it appears that the reabsorption of urea in the proximal tubule takes place almost entirely by back diffusion. The permeability coefficient, P, of the proximal tubules to urea is some 20 times greater than that of the distal convolutions, and in the latter a certain fraction of urea appears to be reabsorbed by solvent drag. In contrast, the reflection coefficient for urea in the distal tubule does seem to be smaller than in the proximal tubule (101).

The reabsorption of urea from tubular fluid would be even greater and its excretion consequently less efficient if it were not exposed to the counter-current mechanism (see page 75) in the renal medulla. As a result of counter-current diffusion in the loops of Henle and the vasa recta, urea continuously diffuses out of ascending into descending limbs. Thus, its concentration is kept high in the structure surrounding the collecting ducts, and the concentration gradient between the fluid in collecting ducts and the rest of the renal medulla is kept relatively small.

Reabsorption of Bicarbonate in the Kidney

If ammonium chloride is administered to an experimental subject for several days, a state of acidosis develops and the bicarbonate concentration in his plasma falls from its normal value of 26 to 28 mM/l. to about 13 mM/l. If then the plasma bicarbonate is slowly restored by the administration of bicarbonate, no bicarbonate is excreted until the threshold concentration of 26 to 28 mM/l. plasma is attained. Only then will bicarbonate be excreted, and it will be excreted in quantities proportional to the amount filtered at the glomeruli (see Fig. 25 and ref. 73). This behavior has already been noted in connection with glucose reabsorption, and just as in the case of glucose, this relationship will hold as long as GFR remains constant, because the maximum reabsorptive capacity for bicarbonate varies directly with the GFR. The measurements shown in Fig. 25 were obtained from three experimental subjects, and in order to make them comparable with one another, the bicarbonate reabsorption in mM/min is divided by the GFR. Only in this way can the characteristic flat portion of the curve, which is seen at high bicarbonate concentrations in the plasma, be reproduced.

Increases in the GFR following meals do not endanger bicarbonate homeostasis, since increased bicarbonate reabsorption keeps pace with the increasing filtered load. This is the more significant since the threshold concentration for bicarbonate is very close to the normal plasma concentration (28 mM/l. in man), and hence there is no reserve of reabsorptive capacity

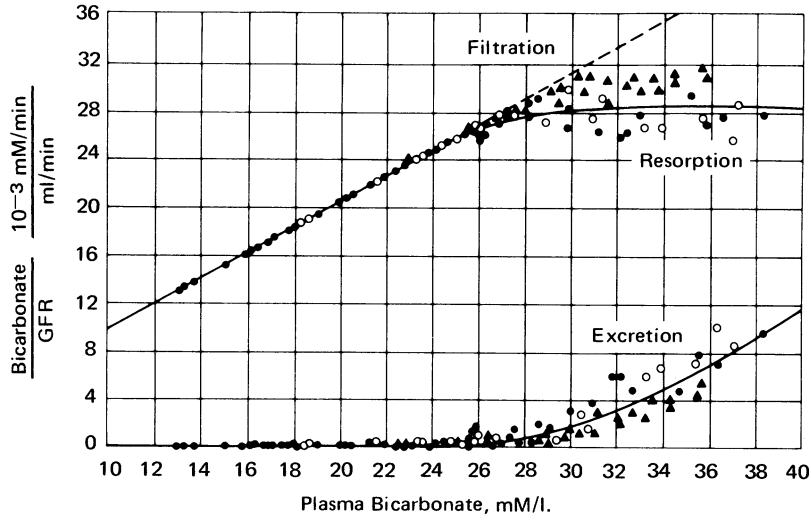


Fig. 25. Relationship between filtered load, tubular reabsorption, and urinary excretion of bicarbonate at constant P_{CO_2} (40 mm) in man. From Pitts et al. (73).

available in the tubules as there is in the case of glucose. At the same time, this means that plasma bicarbonate is kept very constant, an important fact in the regulation of acid-base balance. If a "metabolic alkalosis" develops and is accompanied by a high plasma bicarbonate, the superfluous bicarbonate is excreted as long as the threshold concentration is exceeded.

The bicarbonate threshold is less constant than the glucose threshold. In particular, changes in the partial pressure of carbon dioxide in the plasma (P_{CO_2}) influence bicarbonate reabsorption (75). The threshold concentration of 28 mM/l. depicted in Fig. 25 applies when the P_{CO_2} is 40 mm Hg. Figure 26 illustrates how the threshold increases with increases in P_{CO_2}, with more bicarbonate per milliliter of filtrate being reabsorbed as the P_{CO_2} rises. Figure 26 is based on experiments with dogs in which the P_{CO_2} was altered by varying the CO_2 concentrations in the inspired air. By this means the threshold can be changed in either an upward or a downward direction by a factor of 2. This dependence of bicarbonate reabsorption on the arterial P_{CO_2} is of great importance and affords an explanation of the way the kidney can compensate for acidosis or alkalosis of respiratory origin.

The major portion of filtered bicarbonate is reabsorbed in the proximal tubules, as may be inferred from the following considerations: (1) the pH of tubular fluid falls by about 0.5 of a unit along the course of the proximal convolutions (see Fig. 28); (2) since it is supposed that the tubular wall is freely permeable to CO_2 ,the P_{CO_2} will be about the same in the tubular

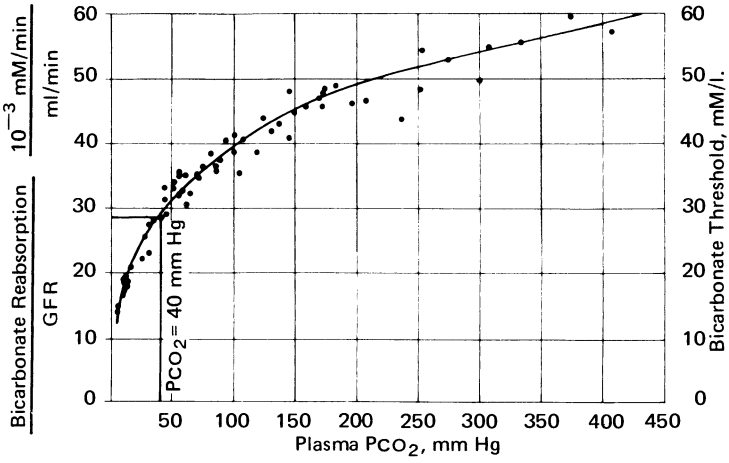


Fig. 26. Relationship between external P_{CO_2}, bicarbonate reabsorption and bicarbonate threshold in the dog. Modified from Rector et al. (75).

fluid as in the plasma and will not change appreciably during the reabsorption of bicarbonate.

At the beginning of the proximal tubule, the pH will be defined by the Henderson-Hasselbalch equation, i.e.,

$$\mathrm{pH} = \mathrm{pK} + \log \frac{[HCO_3^-]_P}{\alpha\, CO_2 \times P_{CO_2}} \tag{17}$$

whence

$$7.4 = 6.1 + \log \frac{26}{0.03 \times 43}$$

At the end of the proximal convolution, the corresponding figures will be

$$6.9 = 6.1 + \log \frac{X}{0.03 \times 43}$$

where

$$X = (HCO_3^-)_{TF} = 8 \text{ mM/l.}$$

Now the filtered load of bicarbonate ($\dot{M}$) will be given by

$$\dot{M} = \mathrm{GFR} \times (HCO_3^-)P = \frac{120 \text{ ml}}{\text{min}} \times \frac{1000 \text{ ml}}{26 \text{ mM}} = \frac{3.12 \text{ mM}}{\text{min}}$$

The bicarbonate remaining in the proximal tubule per unit time, $(\dot{M})_{RTF}$, will likewise be

$$(\dot{M})_{RTF} = \frac{\text{GFR}}{(TF/\text{pinulin})_{RTF}} \times [HCO^-]_{RTF} = \frac{120}{2.5} \times \frac{8}{1000} = \frac{0.38 \text{ mM}}{\text{min}}$$

Thus, about 88 percent of filtered bicarbonate $\left(\frac{3.12 - 0.38}{3.12} \times 100\right)$ must be reabsorbed in the proximal tubule.

Mechanisms of Bicarbonate Transport

The driving force for bicarbonate reabsorption is the tubular secretion of hydrogen ions, which is presumed to take place as in Fig. 27. H^+ ions are secreted into tubular fluid from the tubular cells, and to maintain electrical neutrality, a cation-exchange mechanism occurs at the luminal membrane of the cell. For each H^+ ion leaving the cell, a Na^+ ion is taken up. Within

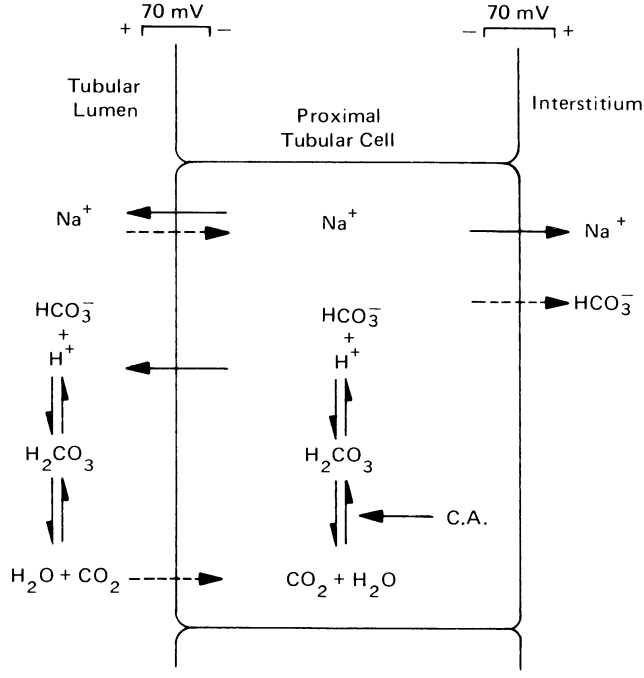


Fig. 27. Mechanism of bicarbonate reabsorption in the proximal convoluted tubule. C.A. = carbonic anhydrase. Modified from Pitts (70).

the tubular lumen the H^+ ions are, together with HCO_3^- ions, in equilibrium with undissociated carbonic acid, which itself is almost entirely present as CO_2 and water. The CO_2 formed in the lumen diffuses into the cells, where it again combines with water to form carbonic acid, this last reaction being greatly accelerated by the presence in the cells of the enzyme carbonic anhydrase. The carbonic acid formed within the cells once more dissociates into H^+ ions and HCO_3^- ions, the former becoming available for secretion. The reabsorption of bicarbonate is especially favored in the proximal tubules by the fact that carbonic anhydrase is located in the brush border of the lining cells. Because of this, carbonic acid formed from the secreted hydrogen ions and filtered bicarbonate is rapidly dehydrated to CO_2 and H_2O at the luminal wall. This leads, on the one hand, to rapid reabsorption of the bicarbonate. On the other hand, it prevents any considerable increase in the concentration of free H^+ ions in the tubular fluid.

In the proximal tubule there exist electrical potential differences between the cell interior and the tubular lumen and also between the cell interior and the interstitium (see Fig. 27). Since the cell interior is negative with respect to the lumen, reabsorption of bicarbonate at this boundary would be taking place against an electrical gradient. This difficulty is circumvented by the conversion of bicarbonate to CO_2, which is uncharged. Transport out of the cell into the interstitium is, however, favored by the electrical gradient. Broken arrows indicate passive movement.

Hydrogen Ion Transport

Secretion of Hydrogen Ions. Tubular cells along the entire length of the nephron and collecting ducts are apparently capable of secreting hydrogen ions, as is illustrated in Fig. 28. In the experiment on which this figure is based, pH was measured in samples obtained by micropuncture from proximal and distal convolutions of nondiuretic rats and in the bladder urine (34). The greatest fall in pH occurs between the termination of the distal tubule and the bladder, i.e., in the region of the collecting ducts (102).

This finding does not conflict with the idea that the quantity of H^+ secreted in the collecting ducts is small compared with the proximal tubules. From the calculation on page 50 it would appear that in man 2.74 (3.12 − 0.38) mM of bicarbonate are reabsorbed each minute by the proximal tubules. For every bicarbonate ion reabsorbed, a hydrogen ion must be secreted. This amounts to about 4000 meq H^+ per day. As further bicarbonate is reabsorbed in the remainder of the nephron, an additional 500 meq per day of H^+ are secreted. It will be recalled that only 1 percent of filtered bicarbonate actually appears in the bladder urine (see Fig. 25). By contrast, the H^+ responsible for the acidification of tubular fluid are

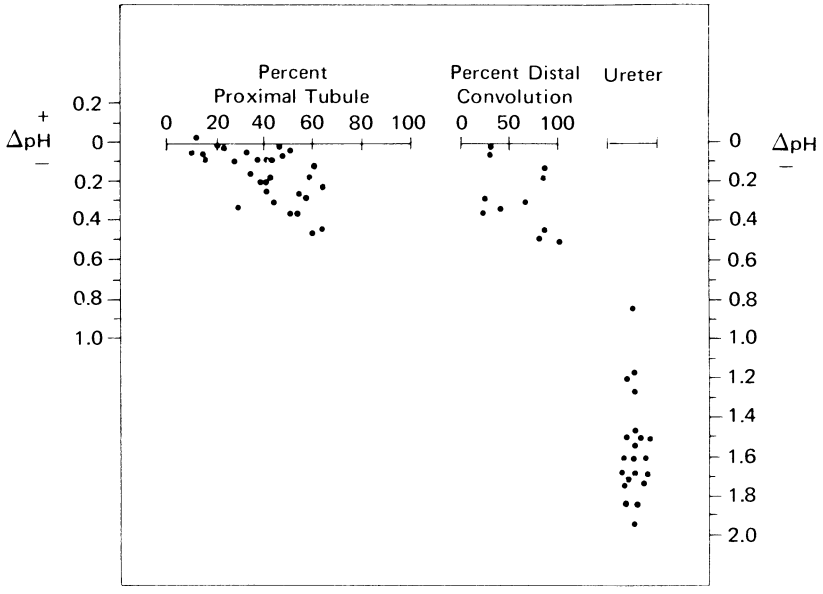


Fig. 28. Decline of the pH of tubular fluid along the nephron, as observed in micropuncture experiments. The site of puncture is indicated as a percentage of the length of the tubular segment. From Gottschalk et al. (34).

insignificant quantitatively, amounting to 0.0065 meq per day in the proximal tubules and in the collecting ducts, 0.12 meq per day.

Since there exists a potential difference between the inside of the proximal tubular cells and both the luminal fluid and the interstitial fluid (Fig. 27), the transport of protons must take place against an electrical gradient.

With a measured pH of 6.9 in the proximal tubular fluid, the consequences of a passive equilibrium can be calculated from the Nernst equation as follows:

$$E = \frac{R \cdot T}{F \cdot Z} \cdot \ln \frac{[H]_{cell}}{[H]_{TF}} \tag{18}$$

or

$$E = 61 \text{ mV } (\log [H]^{+}_{cell} - \log [H]^{+}_{TF})$$

i.e.,

$$E = 61 \text{ mV } (- \text{pH}_{cell} + \text{pH}_{TF})$$

which leads to a value for intracellular pH of 5.75. Although reliable direct

measurements of intracellular pH are not available, this calculated value is improbably low. One must therefore presume that the secretion of H^+ is a case of active transport.

The same considerations that have been advanced with regard to the proximal tubule are equally valid for the distal segments, and more especially for the collecting ducts. While it is true that the potential difference here between cell interior and tubular fluid is only 55 mV, the pH of distal tubular fluid can fall to 4.5.

Excretion of Hydrogen Ions

Of the 5000 meq or so of H^+ that the human kidneys secrete each day, only some 60 meq reach the bladder. This latter figure corresponds to the excess of acid radicals arising from metabolism in a healthy adult man (70). Although a small fraction of the total, even this quantity of acid cannot be excreted in the free form. Since the daily urine volume is about 1.5 l., if 60 meq of strong acid is present in this volume of water, the H^+ concentration would be 40 meq/l. and the pH would be 1.4. Since the pH of the urine cannot fall much below 4.5, the maximum H^+ concentration cannot exceed 0.07 meq/l. Thus, only 0.2 percent of the H^+ produced by metabolism can be excreted in the free form. To deal with the remainder the kidney has two mechanisms at its disposal, *viz.*, the excretion of titratable acid and the secretion of ammonia.

Excretion of Titratable Acid

Phosphoric acid released by the catabolism of proteins and phospholipids is buffered locally with bicarbonate. At a pH of 7.4 this gives rise to secondary phosphate by the following reaction:

$$H_3PO_4 + 2\ Na\ HCO_3 \rightleftharpoons Na_2\ HPO_4 + 2H_2O + 2CO_2$$

The CO_2 thus generated is eliminated via the lungs.

The excretion of phosphate in the form of the secondary salt could lead to an undesirable loss of sodium. The reserves of bicarbonate would soon be exhausted and an acidosis would develop. Because the kidneys can excrete urine at pH as low as 4.5, the buffering capacity of the phosphate ions can be fully utilized by the conversion of the secondary into the primary form:

$$Na_2\ HPO_4 + H_2O + CO_2 \rightleftharpoons Na\ H_2\ PO_4 + Na\ HCO_3$$

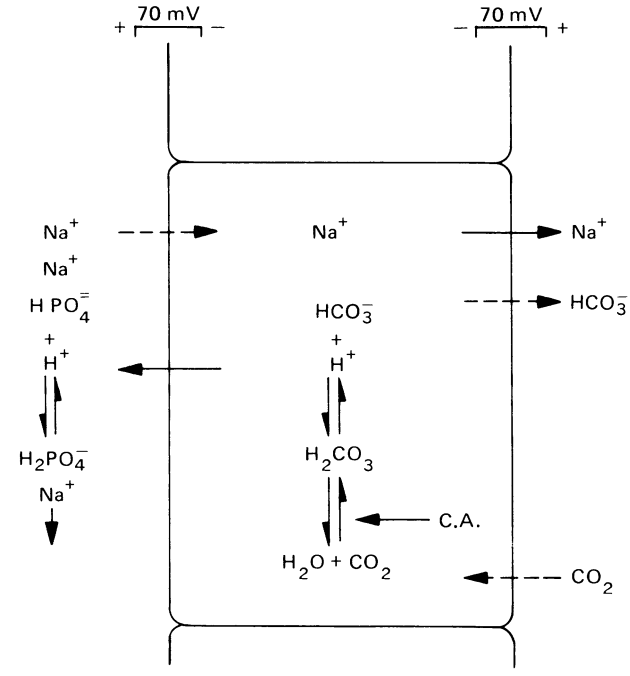


Fig. 29. Mechanism of excretion of titratable acid in the proximal tubule C.A. = carbonic anhydrase. Modified from Pitts (70).

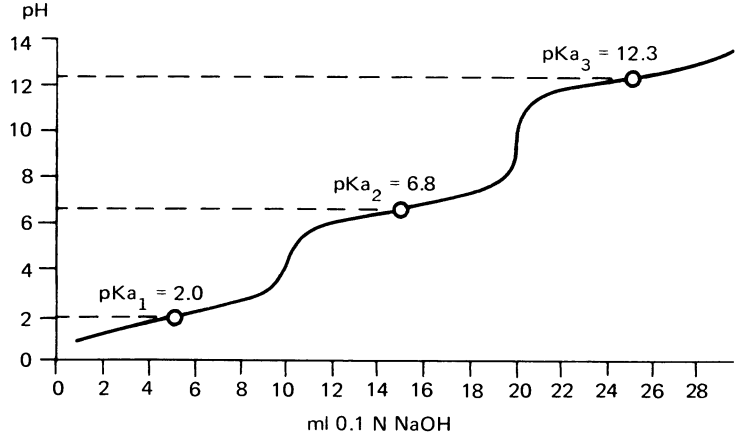


Fig. 30. Titration curve of phosphoric acid (10 ml of 0.1 M) with sodium hydroxide (0.1 N) representing the transitions $H_3PO_4 \rightleftarrows H_2PO^- \rightleftarrows HPO_4^- \rightleftarrows PO_4^{\equiv}$.

Thus, a recovery of bicarbonate is realized along with the elimination of H^+ (Fig. 29).

As is clear from the titration curve of phosphoric acid with sodium hydroxide (Fig. 30), it is only the second dissociation constant which is physiologically important. The pK is 6.8 for this transition of primary $H_2\ PO_4^-$ into secondary HPO_4^{2-}. Using the Henderson-Hasselbalch equation

$$pH = pK + \log\left[\frac{\text{free acid}}{\text{salt}}\right] \tag{19}$$

it is easily calculated that at the normal pH of extracellular fluid, i.e., 7.4, the ratio of secondary to primary phosphate is 4:1. As pH decreases, the proportion of primary phosphate increases so that at a pH of 6.8 it makes up 50 percent of the total, and at the most acid pH attainable in human urine (about 4.5) it accounts for 99.5 percent.

By the conversion of secondary into primary phosphate, between 10 and 30 meq of H^+ can be eliminated from the body, and through this device at least one-half of the bicarbonate expended in buffering can be recovered.

Excretion of Ammonia

As long ago as 1921 Nash and Benedict (64) found that ammonia is formed in kidney cells. It arises from amino acids and more particularly from glutamate by the action of the enzyme glutaminase (71).

According to the Brönsted definition ammonia (NH_3) is a base which in acid solution takes up H^+ with the formation of ammonium (NH_4^+). The free base ammonia is readily soluble in lipids and therefore easily moves out of the cell across the cell membrane into tubular fluid. The "acid" ion ammonium, on the other hand, is very poorly soluble in lipids.

The equilibrium

$$NH_3 + H^+ \rightleftharpoons NH_4^+$$

with a pK value of 9.37 (see page 59) is shifted toward the right as pH decreases. The concentration of ammonium ions will therefore be greatest on the side of the membrane where pH is lowest. In Fig. 28 the existence of a pH gradient between interstitial fluid and the more acid tubular fluid is depicted. Hence, diffusion of ammonia formed in the tubular cells into tubular fluid is favored (see non-ionic diffusion, page 59). By combining

there with H^+, it accumulates as NH_4^+, and this happens more readily the lower the tubular fluid pH. Because of their low solubility in lipids, ammonium ions cannot readily diffuse back out of the tubular fluid and are excreted in the urine. In this way H^+ can be secreted into the tubular fluid and mopped up even at low intra-tubular pH when there may be no bicarbonate present to buffer H^+.

By virtue of the ammonia mechanism, the kidney is enabled to excrete "acidic" anions and at the same time to recover bicarbonate that was being used to buffer them in the extracellular fluid. These anions of strong acids form ammonium salts in the tubular fluid and in this way are excreted:

$$Na_2SO_4 + 2H_2CO_3 + 2NH_3 \rightleftharpoons (NH_4)_2\,SO_4 + 2\,NaHCO_3$$

Ammonia can be produced in all kidney cells that take part in H^+ excretion, as has been demonstrated by micropuncture experiments.

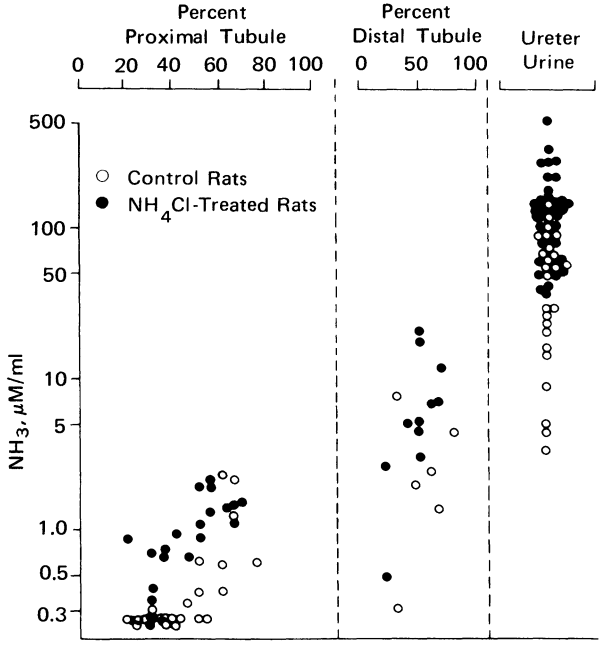


Fig. 31. The rise in ammonia concentration in the tubular fluid with increasing distance from the glomerulus. The data were obtained from micropuncture experiments in normal and acidotic rats. The site of puncture is indicated as a percentage of the length of the appropriate segment, 0 percent representing the beginning. From Glabman et al. (32).

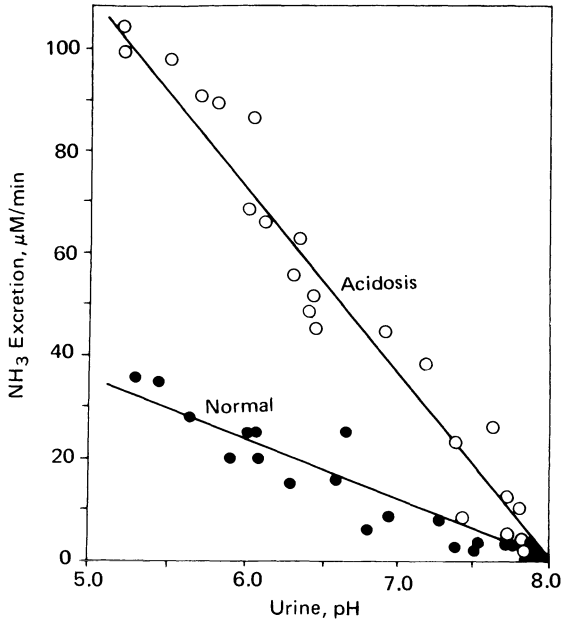


Fig. 32. The relationship between urinary pH and ammonia excretion during conditions of normal acid-base balance and during chronic acidosis. The acute changes of urine pH were brought about by infusing bicarbonate. After Pitts (71).

Figure 31 shows that there is an increase in the concentration of ammonia in proximal and distal tubular fluid and also in the region of the collecting ducts, and this increase is greater than can be accounted for by the reabsorption of fluid in these parts of the nephron (132). The proximal tubule is normally responsible for 50 percent of the ammonia secreted, but in acidosis this proportion can rise to 70 percent of that found in the bladder urine. Under normal conditions 30 to 50 meq per day of acid radicals are excreted as ammonium salts. However, in case of need, e.g., in diabetic keto-acidosis, the quantity may be increased 10-fold. Such an increase in ammonia secretion in cases of acidosis is brought about by non-ionic diffusion as described above and also by increased production of ammonia within the renal cells. Two situations are illustrated in Fig. 32. If urinary pH is raised by the continuous infusion of bicarbonate, an inverse relationship between it and the rate of ammonia excretion becomes apparent, as would be expected from the occurrence of non-ionic diffusion. In addition, in the acidotic animal the rate of ammonia secretion at any given pH is higher than in the normal control animal.

Secretion of Organic Acids

Ever since the experiments of Heidenhain in 1874 (39), it has been known that the kidneys have the ability to secrete a number of different substances. In addition to the quantities of these substances filtered at the glomeruli, additional quantities are extracted from the peritubular blood by cells of the proximal tubules, transported into the tubular fluid and so into the urine. Among such substances are a number of weak organic acids such as para-aminohippuric acid (PAH), diodrast, phenol red, penicillin, sulfonamides, etc. (15). These are all foreign to the animal, not being normal metabolites, so that their excretion by active transport mechanisms is probably accidental.

Specificity of the Transport System for Organic Acids

Recent studies suggest that the transport system for organic acids is required in order to introduce these essential metabolic substrates into the renal cells. It has already been mentioned that, because of their reabsorptive functions, the energy metabolism of the cells of the proximal tubules is very high. Surprisingly, however, they seem to depend for energy production not upon glucose, which is present in the plasma at a relatively high concentration (about 5 mM/l.), but rather upon such substrates as α-oxo-glutarate, lactate, and free fatty acids whose concentration in plasma is relatively low (0.06 mM, 1.3 mM, and 1.0 mM, respectively) (11). Hence, a potent transport system is required to bring these substances into the cells in sufficient quantities. Moreover, the cells seem able to utilize these substances only when they are presented to the basal side of the cell, i.e., the side contiguous to the blood. Apparently, the various foreign acids (e.g. PAH) that are secreted compete for the transport system, which normally carries the naturally occurring substrates (11). In this way they gain access to the interior of the cells, but unlike α-oxo-glutarate and other metabolic substrates, they are not catabolized. Since back diffusion through the basal side of the cell is counteracted by the active transport system, the unnatural compounds come into concentration equilibrium with the tubular fluid and so are excreted.

Utilization of α-oxo-glutarate has been observed in the liver as well as in the kidney, and liver cells are also capable of actively transporting PAH. On the other hand, most cells in the body do not utilize α-oxo-glutarate, and are, as are muscle cells, practically impermeable to this substance; likewise, those cells that are impermeable to α-oxo-glutarate do not take up PAH.

Non-Ionic Diffusion

The individual weak acids differ considerably in the readiness with which they are secreted (109), and it is very probable that this different behavior is related to their physicochemical properties. Weak acids are characterized by the fact that their ionization in solution is depressed by falling pH. The extent of their dissociation is determined by their so-called pK. According to the general buffer equation (Henderson-Hasselbalch equation):

$$\text{pH} = \text{pK} + \log\left(\frac{\text{undissociated acid}}{\text{dissociated salt}}\right) \tag{20}$$

the pK value is equal to the pH at which exactly one-half of the total acid present is in the undissociated form. The ratio following the log symbol then becomes unity, of which the logarithm is zero. (The weaker the acid, the higher is the pK value.)

In the ionized form the weak electrolytes can scarcely penetrate a biological membrane passively because the middle layer of the plasma membrane (see page 30) is composed of hydrophobic lipid, and ionized compounds have a very low solubility in lipids.

However, even in the undissociated form, weak electrolytes exhibit very different solubilities in lipids (89). These differences can be demonstrated *in vitro* by determining the solubility of the various substances in a non-polar solvent such as ether or chloroform and comparing this with their solubility in water. The greater the partition coefficient (Kp), the greater is the solubility of the substance in lipid.

Those weak acids that have the lowest fat-solubility have the highest clearance, so once they have entered the tubular fluid by filtration or

Table 4. pK and partition coefficient (Kp) of a number of weak organic acids that are secreted by the kidney, together with their renal clearances relative to inulin.

Substance	pK	Kp[a]	$\frac{C_x}{C_{\text{Inulin}}}$
Diodrast	2.7	< 0.01	5.16
PAH	3.8	< 0.01	5.11
Salicylic acid	3.0	2.9	1.1[b]
Probenecid	3.4	> 100.0	0.01[b]

[a] Chloroform/water.
[b] At pH 7.4.

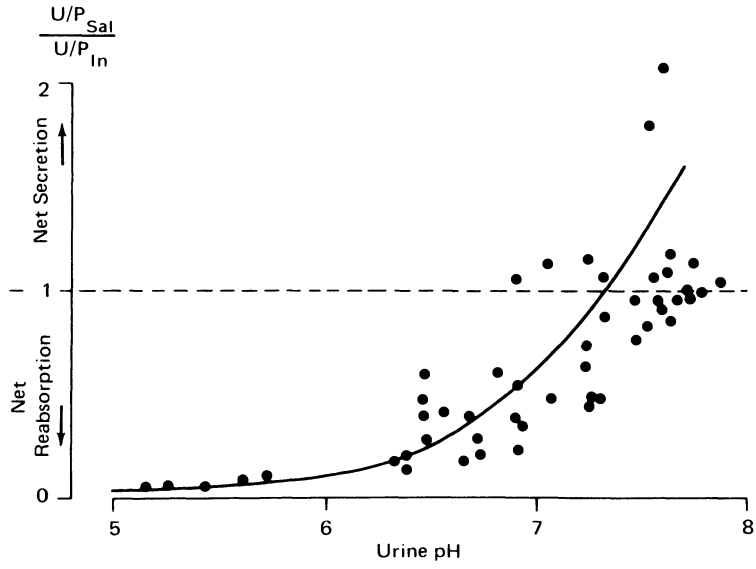


Fig. 33. Net reabsorption (below) and net excretion (above dotted line) of salicylic acid in man, showing the effects of urinary pH. The salicylic U/P to inulin U/P ratio gives the fraction of filtered salicylic acid appearing in urine. (This is an example of non-ionic diffusion of a weak organic acid.) Modified from Gutman et al. (38).

secretion, they remain there and are excreted. On the other hand, substances with high lipid-solubility can leave the tubular lumen by back diffusion. In Table 4 several substances with a similar pK value that are secreted by renal cells are compared with respect to their clearance and their partition coefficients. From the table it appears that a high partition coefficient goes with a low clearance.

While the clearances of PAH and diodrast are independent of urinary pH, the clearances of the lipid-soluble substances vary with the pH of urine. In Fig. 33 this is illustrated for the case of salicylic acid, where the clearances fall off as urinary pH drops, because then the concentration gradient from tubular fluid to interstitium for the non-ionized acid is highest when the pH is low, and hence reabsorption by back diffusion is favored.

Practical use is made of the process of non-ionic diffusion in the treatment of barbiturate poisoning. Undissociated barbituric acid has a high lipid solubility, and hence a tendency to back-diffuse out of tubular fluid into the less-acid extracellular fluid. If, therefore, ionization is encouraged by preventing acidification of the urine (which can be done by infusing

bicarbonate) and if at the same time the reabsorption of filtered fluid is reduced by administering a diuretic drug, so lowering the concentration gradient for barbituric acid, the elimination of the latter will be greatly accelerated.

Renal Extraction

As a result of secretion by the tubules, as much as 90 percent of PAH or diodrast in renal arterial blood can be extracted, as long as the concentration in the blood is low (less than 5 mg %). The concentration in tubular fluid may then exceed that in plasma five-fold. At high plasma concentrations, the extraction ratio falls. The extraction ratio for any substance is given by its arterio-venous concentration difference across the kidney, expressed as a fraction of its concentration in arterial blood.

For a long time it was thought that the entry of weak acids into the tubular fluid was limited by a transport maximum, i.e., by a maximum rate at which the cells could transport the material (86). Recently, however, it has been shown that there is a limit to the concentration that an organic acid can attain in the tubular cells, and hence in the tubular fluid. It is this concentration limitation that sets a limit to the quantity secreted per unit of time (19).

The extraction ratio thus depends on the relationship of plasma concentration (which also determines the concentration in the glomerular filtrate) to the maximum attainable concentration in the tubular cells. On the other hand, the filtration fraction (FF) is also decisive in that it represents the fraction of plasma flowing into the kidney (RPF), which is filtered at the glomeruli and thus becomes available to accept the secreted weak acid. One can write the following equation to describe the relationship between a falling extraction ratio and a rising plasma concentration of any substance:

$$E = \frac{TF_m}{P} \times FF \tag{21}$$

where E = extraction ratio
FF = filtration fraction
P = concentration of substance in the plasma
TF_m = the maximum attainable concentration of the substances in the tubular fluid.

Equation (21) is illustrated graphically in Fig. 34, from which it may be seen that when the plasma concentration becomes equal to the maximum attainable concentration in the tubular fluid, no further increase in net secretion will take place. Any additional excretion of the substance with

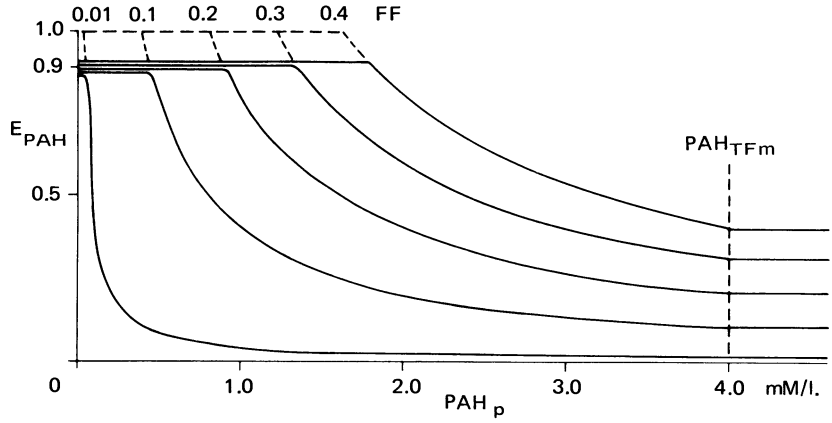


Fig. 34. Extraction of PAH from the renal plasma showing its dependence on plasma concentration (PAHp) and also the effects of changes in the filtration fraction (FF).

further increase in plasma concentration can occur only by glomerular filtration; its clearance value will become equal to the inulin clearance, and the extraction ratio will be determined solely by the filtration fraction.

These arguments will apply only as long as a substance like PAH does not leave the tubular fluid (by back diffusion). If a substance is subject to non-ionic diffusion, the extraction ratio can fall to a very low value and can actually become zero. Furthermore, if the transport mechanism is depressed by metabolic inhibitors—such as cyanide or dinitrophenol, by lack of oxygen, or by disease processes affecting the tubular cells—the extraction ratio is reduced.

Even substances with the highest extraction ratios, such as PAH, are not completely extracted, and several explanations of this have been put forward. One possibility is that a part of the renal blood flow may perfuse areas of the kidney where no extraction takes place. Another possibility is that there is competitive inhibition from the normal substrates of renal metabolism that are seeking entry into tubular cells.

Measurement of Renal Plasma Flow

The fact that certain weak acids undergo 90 percent extraction is utilized for the measurement of renal plasma flow (RPF). Because of the ease with which it can be estimated, PAH has been extensively employed. The clearance of PAH calculated as follows:

$$C_{PAH} = \frac{U_{PAH} \times \dot{V}_u}{P_{PAH}} \tag{22}$$

gives a reasonable approximation of renal plasma flow because this clearance represents the volume of plasma which is freed or "cleared" of PAH per unit time.

For precise measurements of the RPF using PAH, the extraction ratio should also be determined. This is especially important in the clinical investigation of diseased kidneys, in which the extraction ratio is reduced, when the renal plasma flow is to be measured for diagnostic purposes. When the extraction ratio has been measured, the following equation applies:

$$\text{RPF} = \frac{C_{\text{PAH}}}{E_{\text{PAH}}} = \frac{U \times \dot{V}_u \times Pa}{Pa\,(Pa - P_R)} = \frac{U \times \dot{V}_u}{Pa - P_R} \tag{23}$$

where U = PAH concentration in the urine
Pa = PAH concentration in the arterial blood
P_R = PAH concentration in the renal vein
$\dot{V}_u$ = volume of urine produced per minute

It will be noticed that this formula is based on an application of the Fick principle, which is also used in the determination of the cardiac output from oxygen consumption and arterio-venous oxygen differences.

For even more exact measurements, account must be taken of the fact that the volume of blood leaving the kidneys is slightly less than the volume entering them, the difference being the volume of urine excreted. Since the quantity of substance entering the system must be equal to the total leaving it, the following equation must hold:

$$P_a \times \text{RPF} = P_R\,(\text{RPF} - \dot{V}_u) + U \times \dot{V}_u$$

whence

$$\text{RPF} = \dot{V}_u \frac{U - P_R}{P_a - P_R} \tag{24}$$

If the RPF is known and also the hematocrit (Hct), the total renal blood flow (RBF) is given by:

$$\text{RBF} = \frac{\text{RPF}}{1 - \text{Hct}} \tag{25}$$

Renal Hemodynamics

Magnitude of the Renal Blood Flow. The two kidneys of an average 70-kg man weigh altogether about 300 g, and thus constitute about 0.4

percent of the body weight. They receive about 25 percent of the cardiac output, i.e., about 1200 ml of blood per minute.

This high blood flow is required not so much to provide for the metabolic demands of the kidneys (since the available oxygen and other substrates are never fully utilized), but rather to provide the large volume of fluid to be filtered at the glomeruli (see page 12). A high filtration rate is essential for the kidneys to fulfill their role of reprocessing the extracellular fluid and eliminating unwanted end products of metabolism and any superfluous water or other ingested material. In accordance with local functional requirements, blood flow is very unevenly distributed within the kidney. The cortex, where filtration occurs, receives the highest rate of blood flow per unit weight of any organ in the body. Before we comment further on this feature, it is necessary to describe briefly the anatomy of the renal vasculature.

Vascular Anatomy of the Kidney

As a general rule each kidney is supplied by a single renal artery. This artery arises directly from the aorta, and at some point before or on reaching the hilus of the kidney, it divides into two or more branches. These pass dorsally and ventrally across the renal pelvis and then subdivide into the interlobar arteries from which the arcuate arteries arise and course more or less parallel to the surface of the kidney in the boundary region between cortex and medulla. From the arcuate arteries the interlobular arteries arise and pass outward toward the surface of the organ. Finally, the afferent arterioles are given off from the interlobular vessels and break up into the capillary loops of the glomeruli (see Fig. 35).

The outflow from the glomerular capillaries passes into the efferent arterioles, which leave the glomerulus alongside the afferent arterioles at the vascular pole. The efferent arteriole of the superficial cortical glomeruli breaks up again into another capillary network, the peritubular vessels. Thus, in the kidney there are two capillary beds arranged in series. In the renal cortex the second of these beds envelops the proximal and distal convoluted tubules.

The vessels that supply the medulla arise from relatively thick efferent arterioles emerging from the so-called juxta-medullary glomeruli. They divide at the outer margin of the outer medullary zone into the arterial vasa recta and run in characteristic parallel bundles through this zone (Fig. 35). Before reaching the boundary of the inner medullary zone, most of the vasa recta have already divided into fine capillaries from which the outflow passes into the nearby bundles of the venous vasa recta. The arterial vasa recta which occupy the centers of the bundles pass on into the inner zone

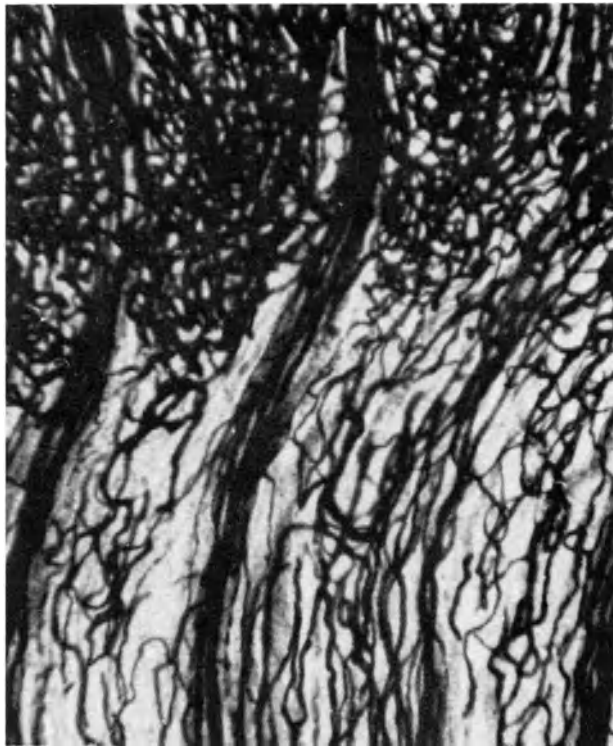


Fig. 35. Section of the outer medullary zone of a rat kidney. The arterial vasa recta and capillary plexuses arising from them have been displayed by an intra-arterial injection of colored neoprene. From Moffatt and Fourman. (63).

of the medulla and divide successively into capillaries from which the blood is returned to the venous vasa recta. (Vasa recta simply means "straight vessels.")

The length of the vasa recta is quite unusual; the capillaries in most organs are no longer than 0.5 mm, whereas those in the renal medulla may be as long as 40 mm. The hemodynamic and functional consequences of this unusual arrangement will be discussed in connection with the mechanism for regulating urine concentration (see page 80).

The venous drainage is arranged in a manner similar to the arterial inflow. In the region of the arcuate veins and interlobular veins there are numerous anastomotic channels. In this respect the venous system of the kidney differs from the arterial system in which no anastomoses are found. Thus, all the branches of the renal artery fall into the class of so-called end arteries. Recent studies also suggest that there are no arterio-venous

anastomoses, and hence there is no direct pathway by which blood from the renal arteries can circumvent the glomerular and peritubular capillary network (51).

Localization of the Renal Vascular Resistance

Figure 36 is a schematic representation of the pressure gradients from the renal artery to the renal vein in the antidiuretic state. The fact that blood flowing through the kidney must traverse two successive capillary beds, each preceded by a muscular arteriole, provides steep hydrostatic pressure gradients. The steepest gradients, and hence the highest resistances, are found across the afferent and efferent arterioles of the glomeruli. These two resistances are matched so that the filtration pressure is maintained as constant as possible. The resistance in the efferent arterioles acts to support the glomerular capillary pressure; at the same time the resistance in the afferent vessels ensures that the glomerular capillaries are protected from fluctuations in the systemic arterial pressure.

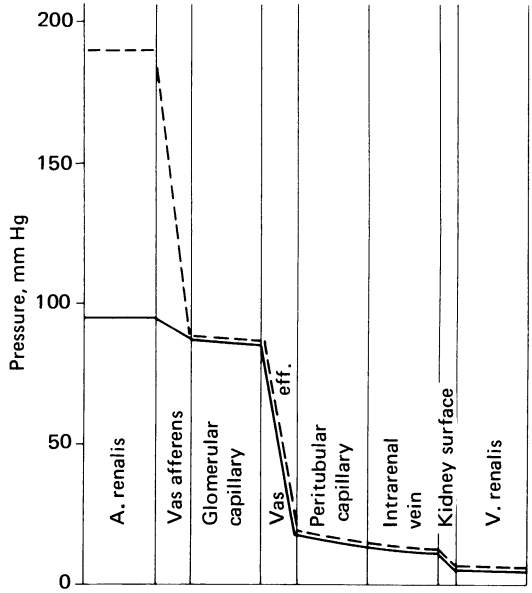


Fig. 36. The stepwise fall in hydrostatic pressure between renal artery and vein when the arterial pressure is between 190 mm Hg (— — —) and 95 mm Hg (————) in the antidiuretic state. As long as arterial pressure is within this range, glomerular and post-glomerular pressures are held constant by the varying resistance of pre-glomerular vessels (vasa afferentia). From Thurau and Wober (99).

As long as the arterial pressure remains between 100 and 190 mm Hg, the filtration pressure, and hence the glomerular filtration rate, is kept nearly constant.

Regulation of Renal Blood Flow

As a result of adjustments in afferent arteriolar resistance in response to variations in the systemic arterial pressure, both the GFR and the renal blood flow are constant. This auto-regulatory behavior is present both in innervated and denervated kidneys and in isolated kidneys, artificially perfused. It appears that under normal conditions, the renal vessels are not subject to sympathetic tone, and therefore there must exist an intrinsic mechanism responsible for adjusting the pre-glomerular resistance to arterial blood pressure. Substances such as papaverine, cyanide, or cocaine, which paralyze the smooth muscle of blood vessels, abolish the auto-regulation of the GFR and the RBF. Fluctuations in arterial blood pressure are then accompanied by fluctuations in blood flow.

These findings have given rise to the notion that the alterations in pre-glomerular resistance are of myogenic origin. A possible stimulus that may be responsible for inducing contraction of the arteriolar smooth muscle when arterial blood pressure rises would be a stretching of the vessel wall. The tension in the wall (T) is given by Laplace's Law, which states that

$$T_{\text{wall}} = r\,(P_{\text{inside}} - P_{\text{outside}}) \tag{26}$$

where r is the internal radius of the vessel, P_{inside} is the intraluminal pressure, and P_{outside} is the pressure in the interstitial fluid surrounding the vessel. The Hagen-Poiseuille Law states that the radius is proportional to the fourth root of the resistance; hence, in considering changes in vascular resistance, changes of r can be neglected as a first approximation, so that the tension in the wall is principally dependent on the transmural pressure, i.e., $P_{\text{inside}} - P_{\text{outside}}$. When arterial pressure rises, the pressure in the peritubular capillaries and also the intrarenal pressure remain constant. The transmural pressure and hence also the tension in the wall of the afferent arterioles must increase when the arterial pressure rises, and this could thus bring about a contraction of these vessels (93).

Although numerous experimental observations lend support to this theory, it appears that an intrinsic vascular response is only part of the explanation of the auto-regulatory control of the GFR. The finding that the phenomenon of auto-regulation is much less prominent in sodium-loaded than in sodium-deficient animals has directed attention to the renin-angiotensin system, since renin production in the kidney is known to be related to the sodium balance of the animal (37). Renin is an enzyme

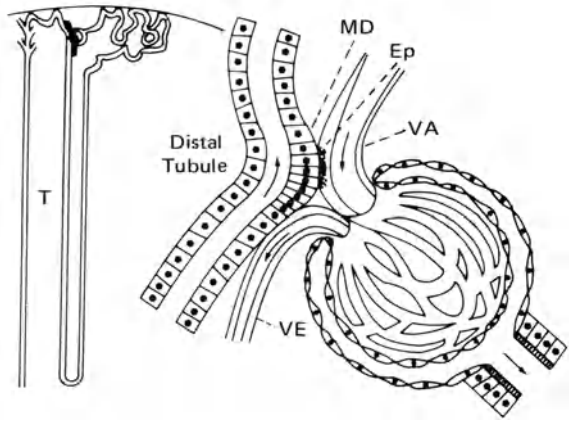


Fig. 37. Semi-diagrammatic drawing of the juxta-glomerular apparatus. T = thick ascending limb of Henle's loop; MD = cells of the macula densa; VA = vas afferens; VE = vas efferens; Ep = epithelioid cells.

that liberates the decapeptide angiotensin I from an α-globulin produced in the liver (and present in the blood plasma). A specific serum peptidase (the converting enzyme) converts angiotensin I into the octapeptide angiotensin II, a very powerful vasoconstrictor agent. Angiotensin II is finally destroyed by further fragmentation under the influence of angiotensinase. The site of renin-production in the kidney is the juxta-glomerular apparatus, found at the point of junction of the thick ascending limb of Henle's loop with the distal convoluted tubule. At this point the tubular cells are in close apposition to the vascular pole of the glomerulus belonging to the same nephron (Fig. 37). At the point of contact the tubular cells have a characteristic columnar shape; these make up the so-called macula densa, while the adjacent cells of the medial layer of the afferent arteriole assume an epithelioid appearance with a granular cytoplasm. These epithelioid cells are thought to be responsible for renin production. The macula densa and the epithelioid cells together constitute the juxta-glomerular apparatus.

As has already been mentioned, the sodium concentration in the fluid reaching the end of Henle's loop is lower than that in the plasma. It has been shown by microperfusion of Henle's loop that the sodium concentration in the early distal tubule varies with the rate of flow of fluid along the loop (12). The greater this flow rate, the greater is the sodium concentration in the early distal tubule. The sodium concentration of fluid reaching the macula densa—or perhaps the rate of sodium reabsorption —appears to be a decisive factor in the auto-regulatory process, since both show a clear correlation with the GFR. The higher the sodium con-

centration at the macula densa and the more nearly it approaches plasma concentration, the lower the GFR becomes. It has even been possible by raising the sodium load at the macula densa of a single nephron to stop filtration altogether in the corresponding glomerulus (98). This and other experimental evidence have made it necessary to consider another autoregulatory mechanism based on a feedback coupling with sodium load (98). This might operate as follows: Suppose that the GFR increases, perhaps as a result of a rise in arterial pressure. This would lead to an increased volume flow through the loop of Henle and so to an increased sodium load at the macula densa. If then one postulates a "sodium receptor" located at the macula, this could provoke an increase in renin release from the nearby epithelioid cells lying in the wall of the afferent arteriole. This would lead in turn to an increased, on-the-spot production of angiotensin I with conversion and liberation of angiotensin II. This, acting on the afferent arteriole, would cause it to constrict and so bring about a reduction in the filtration rate. On the basis of this theory, the constancy of the renal blood flow in the face of changing arterial pressure becomes an incidental and secondary effect. The primary event is the maintenance of a constant GFR, and along with this, the presentation of a constant filtered load of sodium for reabsorption by the tubules.

The Arterio-Venous Oxygen Difference Across the Kidney

Reference has already been made in the discussion of tubular transport to the connection between oxygen consumption and the energy requirements of the transport process. Over and above a basal metabolic rate, oxygen consumption of the kidneys rises linearly with reabsorptive work. This is devoted mainly to the reabsorption of filtered sodium, of which, under normal conditions, about 99 percent is returned to the peritubular blood. A rise in renal blood flow will lead to an increase in filtered sodium and hence in the oxygen requirement of the tubules (48).

This relationship between blood flow and oxygen consumption is a peculiar feature of the kidney, for in this organ oxygen consumption rises and falls with blood flow. The result is that the A-V oxygen difference across the kidney remains nearly constant despite fluctuations in blood flow (Fig. 38). Because of the exceptionally high rate of blood flow, the renal A-V oxygen difference is only 1.5 vols %—much less than that across the systemic circulation as a whole—whereas the average is between 4 and 5 vols %. Since the oxygen consumption of the kidneys depends on renal blood flow, the blood flow can be reduced to about one-third its normal value before any evidence of oxygen-lack, as indicated by a rising A-V difference, becomes apparent.

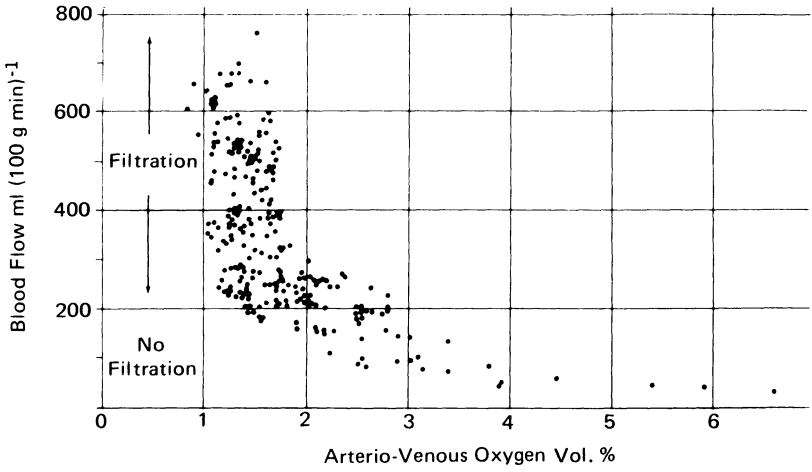


Fig. 38. The relationship between arterio-venous (A-V) oxygen difference and blood flow in the dog kidney. A-V difference remains almost constant as long as filtration continues. Modified from Kramer and Deetjen (48).

The proportionality between blood flow and oxygen consumption holds only as long as glomerular filtrate is being formed. If, at very low blood flows, filtration should cease, the oxygen consumption becomes independent of blood flow. In this state to maintain the basal level of oxygen consumption required for the preservation of structural integrity, the blood becomes more unsaturated than normal. The renal blood flow and A-V oxygen difference then exhibit the reciprocal relationship (Fig. 38) seen in other organs.

The Intrarenal Distribution of Blood Flow

Table 5 presents a summary of the distribution of renal blood flow between cortex and outer and inner medullary zones in the dog. This reveals that the cortex receives on the average about 458 ml/min/100 g, while the outer medulla receives only one-quarter of this quantity, and the inner medulla only about one-sixteenth. Since roughly 70 percent of each kidney is composed of cortical tissue, whereas the outer and inner medullary zones make up 20 percent and 10 percent, respectively, it is clear that the two medullary zones together receive only a small fraction of the total volume of blood flowing into the kidney, *viz.*, 6.5 percent in the case of the outer medulla and a mere 1 percent in the case of the inner medulla.

The given figures in Table 5 apply to the kidney of the dog. The measurements were made (50) by placing microphotometers on the cortex and in the outer and inner medulla, and recording dye-dilution curves at

Table 5. Intrarenal distribution of blood flow, together with data on the mean circulation time and vascular volume in the cortex and the outer and inner medullary zones of the kidney in the dog.

	Weight (percent of whole kidney)	Vascular volume		Time, min	Intrarenal blood flow		
		ml/100g tissue	ml/100g kidney		ml/100g tissue/ min	ml/min 100 g kidney	Percent of total blood flow
Cortex	70	19.2	13.5	0.021	458	321.0	92.5
Outer medullary zone	20	19.2	3.8	0.086	112	22.4	6.5
Inner medullary zone	10	22.0	2.2	0.374	29[a]	2.9	1.0
	100		19.5			346.3	100.0

[a] For this determination circulation time was doubled to correct for the fact that our photoelectric sensor in the papilla measured dye-passage in both ascending and descending vasa recta.

each of these sites, following the injection of a slug of dye into the renal artery (see Fig. 39). The data in Table 5 show clearly the differences between the transit times (E) in the different regions. According to the formula:

$$I = \frac{0.5 \cdot V}{E} \text{ (ml/100 g/min)} \tag{27}$$

the blood flow (I) is inversely proportional to the mean circulation time ($\bar{t}$). The intravascular volume (V) amounts to about 20 ml/100 g tissue in all three regions of the kidney (Table 5).

Almost identical results to those obtained by the photometric method were arrived at by using the radioactive krypton wash-out method in anaesthetized dogs. In this procedure ^{85}krypton is injected through a catheter into the renal artery and the γ-emission is monitored by means of a counter placed over the body surface. The slope of the curve relating wash-out of the isotope to time gives a value for the blood flow based on the following relationship:

$$I = K \cdot \frac{\lambda}{P} \cdot 100 \qquad \left[\frac{\text{ml}}{100 \text{ g/min}}\right] \tag{28}$$

where K = the slope of the wash-out curve
λ = the partition coefficient for ^{85}Kr between blood and tissue
P = the thickness of the organ.

In contrast to organs in which blood flow is homogeneously distributed,

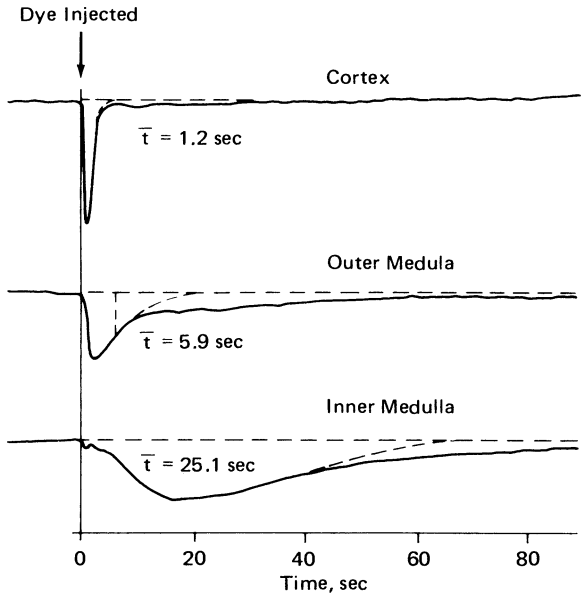


Fig. 39. Dilution curves recorded from the renal cortex and the outer and inner medullary zones following injection of dye into a renal artery. As a result of recirculation of the dye, the apparent transit time was an unduly prolonged solid line, and a correction for this was made by extrapolation from the initial portion of the curve (broken line). $\bar{t}$ = mean capillary transit time. From the original records of Kramer and Deetjen.

the wash-out curve does not show a single exponential decay. It is represented rather by the sum of three or four exponential terms which can be related to the different rates of blood flow through the three regions of the kidney and the perirenal fat.

The krypton method has also been used in human subjects, with results very similar to those obtained in the dog (54). It should be noted that the interpretation of wash-out curves for the medulla is complicated by the fact that the freely diffusible gas is subjected to counter-current exchange (see page 84) between the descending and the ascending limbs of the vasa recta. As a result of this, there will be a short-circuiting of ^{85}Kr in the outer medulla, and only a fraction of the isotope inflow will reach the tips of the papilla.

Medullary Blood Flow

When, in the foregoing discussion, medullary blood flow was described as being small, it must be understood that this only means that it is small by comparison with cortical flow. In absolute terms the medullary blood

flow of 30 ml/100 g/min is 10 or more times greater than that in resting muscle and it is as much as one-half of that of the brain.

The relatively low blood flow through the renal medulla is not a result of there being fewer blood vessels than are present in the cortex; as indicated in Table 5, the volumes of the vascular beds in the medulla and cortex are not vastly different. The cause of the low medullary flow rate is to be found rather in the inordinate length of the perfusion pathway, which introduces a considerable resistance; in addition, it must be noted that during its passage through the medulla the blood undergoes some increase in viscosity. In a "concentrating" kidney (i.e., during antidiuresis) the plasma proteins become more concentrated in the descending limbs of the vasa recta as a result of the withdrawal of water when the blood comes into osmotic equilibrium with the hypertonic milieu near the papilla. Since blood in the ascending limbs of the vasa recta takes up water as it passes from the papilla into the isotonic milieu at the corticomedullary boundary, there occurs what amounts to a short-circuiting of water.

If an individual changes from a state of water restriction to one of water diuresis, the inner medullary blood flow may be nearly doubled (97) and this may be attributable to the fact that the increase in viscosity of the blood traversing the medulla under these conditions is much less than in the antidiuretic state. It is also relevant that the extent of short-circuiting of water is somewhat diminished by the increase in blood flow and so a greater proportion of the blood water must traverse the long pathway through the deeper layer of the medulla.

A further peculiarity of the medullary circulation is its passive behavior in response to changes in blood pressure, whereas with changes in arterial pressure from 90 to 200 mm Hg the total renal blood flow remains virtually constant (see page 67). The medullary blood flow does in fact increase (95) with rising arterial pressure, but this is obscured in measurements of the total renal blood flow, since 90 percent or more of the increase is in cortical flow. This difference in behavior is mirrored in the morphological difference between the cortical glomeruli and the juxta-medullary glomeruli from which the medullary vessels take their origin. The vasa afferentia and the vasa efferentia of the juxta-medullary glomeruli have much less smooth muscle in their walls than do the equivalent vessels in the cortex, and the renin content of their epithelioid cells (see page 68) is very low (69). Also, direct channels connecting the afferent and efferent arterioles can be distinguished in this zone (57).

Urine-Concentrating Mechanism

The human kidney can concentrate urine to an osmolality four times that of plasma. It has become customary to designate the ratio of the

concentrations of osmotically active substances in urine and plasma by the expression U/P_{osm}. Thus, the human kidney is capable of attaining a U/P_{osm} ratio of 4. Animals that live in arid environments where water is scarce may reach a U/P_{osm} ratio of 20. Conversely, animals such as the beaver, which live in or near plentiful supplies of fresh water, tend to pass rather dilute urine, with a U/P_{osm} ratio of less than 2.

Importance of the Medulla

As long ago as 1927 Crane (13) noted that animals such as the frog, whose nephrons possessed no loop of Henle, were not capable of producing concentrated urine. There exists, as can be seen from Fig. 40, a very close correlation between the maximum concentrating ability of a kidney and the relative thickness of the medulla, i.e., the ratio of the medullary thickness to that of the cortex.

In 1942 Kuhn and Ryffel (53) proposed that the urinary concentrating operation was a function of the medulla—where the hairpin shape of Henle's loops and the parallel alignment of the collecting ducts could mediate the concentrating process through a counter-current mechanism. According to Kuhn's theory, the epithelial cells lining Henle's loop build up and maintain a small osmotic gradient between the descending and ascending limbs. This small difference in osmotic pressure is only a fraction

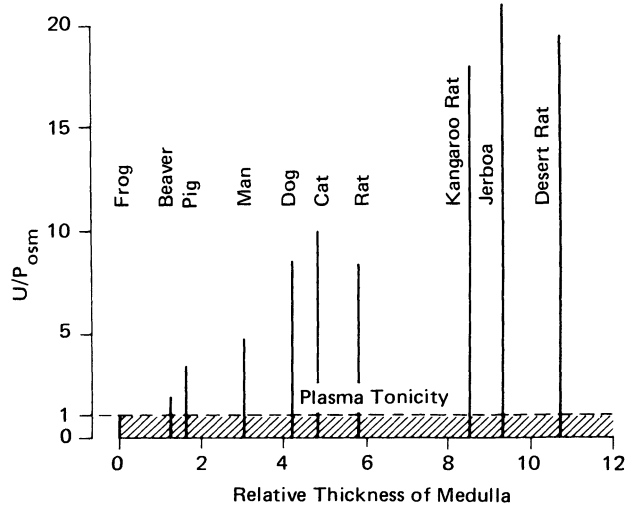


Fig. 40. The relationship between urinary concentrating ability and thickness of the medulla relative to the cortex in kidneys of different animals. Modified from O'Dell and Schmidt-Nielsen (65).

of the difference between plasma and bladder urine, but it can be established at each level of the loop. Because of the movement of the tubular fluid in opposite directions in the two limbs of the loop, a small "single effect" at each level can lead to a large overall effect on the concentration gradient between the base and the apex of the loop, i.e., the "single effect" is multiplied.

Principle of a Counter-Current Multiplier

A simple model (Fig. 41(a)) may be used to describe how a concentration gradient can be built up in a counter-current system. Suppose that a water-impermeable membrane (m) is interposed between the two limbs, S_1 and S_2, of a channel bent acutely in hairpin fashion. Suppose further that this membrane is capable of transporting solute from S_2 to S_1 until a certain limiting concentration difference is reached at each horizontal level.

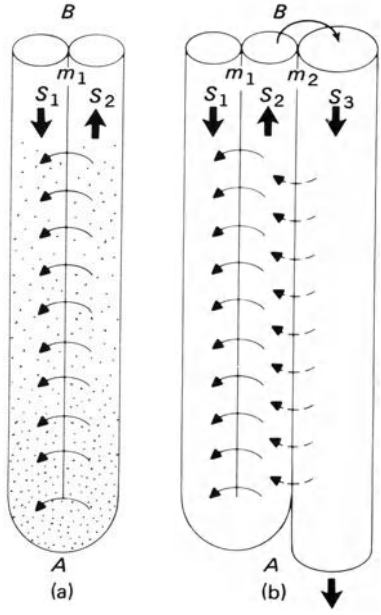


Fig. 41. (a) Model of a hairpin counter-current system in which the membrane (m_1) between the two limbs is capable of actively transporting solute from S_2 to S_1. (b) Model of a counter-current system with the capability for net concentration of solute. The membrane (m_2) between S_2 and S_3 is permeable to solvent, and so the fluid in S_3 comes into osmotic equilibrium with the fluid in S_2. Hence, the fluid emerging from S_3 will have the same osmolarity as that in S_2 at the bend of the hairpin A.

The creation of this concentration difference at a particular level is called the single effect. If now fluid is caused to flow downward in S_1 and upward in S_2 bringing higher concentrations to the transport sites in S_2 this movement will cause a multiplication of the single effect as indicated by the density of the stippling in Fig. 41(a). The osmolarity of the solutions in S_1 and S_2 at the apex (A) of the hairpin will therefore be much greater than at the base (B).

For better understanding the interplay between the single effect and movement of the fluid column, the process is set out in discrete steps in Fig. 42. Here the single effect and the fluid movement, which normally occur simultaneously, are depicted as occurring alternately. Initially (panel 1), the fluid throughout the system has an osmolarity of 300 mosm/l. The single effect then generates a concentration difference of 200 mosm/l. (panel 2), so that the solute concentration in S_1 rises to 400, while that in S_2 falls to 200 mosm/l. In panel 3 fresh fluid with the original osmolarity of 300 mosm/l. has entered S_1 in sufficient quantity to displace one-half its contents into S_2. Once more the single effect comes into operation to generate a gradient of 200 mosm/l., and the situation depicted in panel 4 will result. Note that these three steps have already created a concentration of 500 mosm/l. in the right-hand portion of the hairpin, a greater concentration than could be achieved by the single effect alone. Further entry of fresh fluid into S_1 will lead to the concentration profile seen in panel 5, and a continuation of the same sequence of events will generate a concentration of 700 mosm/l. at the extremity of the hairpin, as in panel 8. It will be noted that the proportion of fluid moving on is progressively less; in panel 3 it is one-half of the volume in S_1; in panel 5 it is one-quarter; and in panel 7, only one-eighth. If individual steps in the progression become infinitely small, we have a continuous process in which osmolarity will rise exponentially from base to apex.

In the model as so far described the fluid flowing from S_2 has a lower osmolarity than that entering S_1. The fluid is concentrated temporarily at the apex of the hairpin because solute is transported from S_2 to S_1. The work done in concentrating this solute can be harvested if a third channel (S_3 in Fig. 41) is added, which permits osmotic equilibrium across the semi-permeable membrane (m_2) between its own contents and the contents of S_2. Fluid flowing down S_3 from B to A loses water continuously, since it is equilibrating with fluid of increasing osmolarity in the neighboring channel S_2, and so it leaves S_3 at A with an osmolarity approaching that at the tip of the hairpin.

Although all minutiae of the concentrating process in the kidney are not yet fully elucidated, it is quite clear that the conditions in the kidney approximate very closely those in Kuhn's model. In the renal medulla the collecting ducts run parallel to Henle's loops, with their characteristic hair-

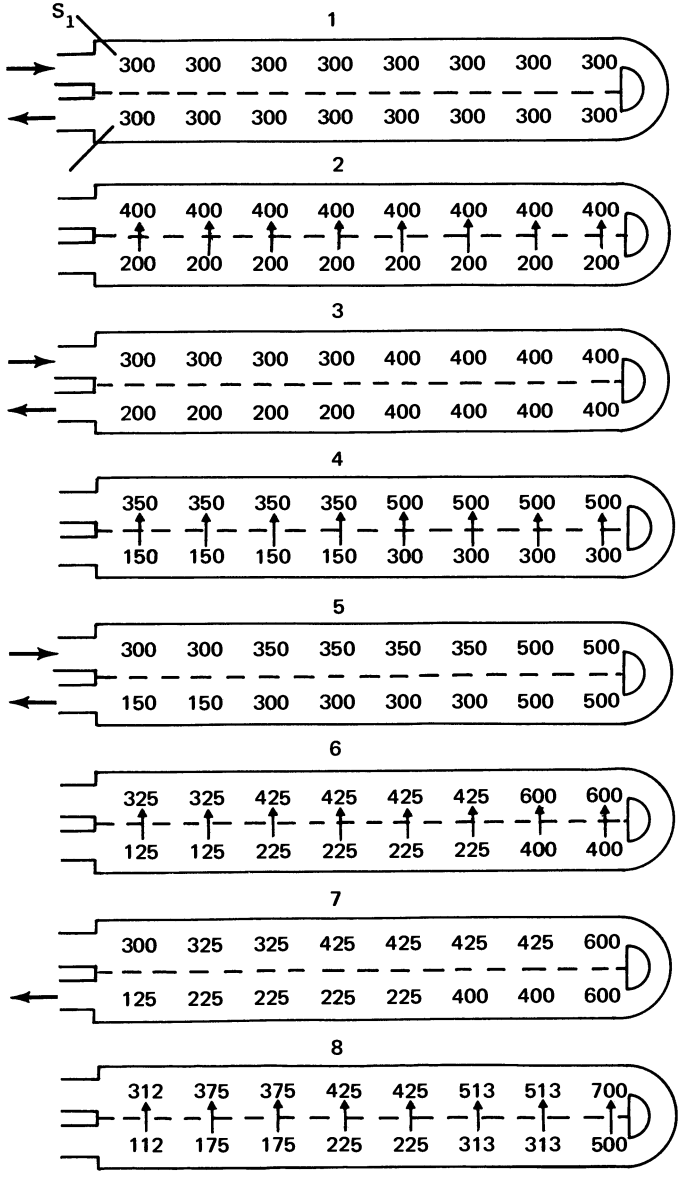


Fig. 42. Schematic representation of the mode of development of a concentration gradient between the beginning and the bend in a hairpin countercurrent system by the multiplying of single effects. For the sake of clarity, the single effect and the onward movement of the fluid are treated as if they occurred in alternating steps, but in reality both processes would take place continuously. Odd-numbered loops show result of flow. Even-numbered loops show result of single effect. After Pitts (70).

pin conformation, and so correspond very well to S_3 in the model. In order for the counter-current concentrating process to function in the kidney, it is not essential that S_1 and S_2 on the one hand and S_2 and S_3 on the other be separated by common membranes. Thin layers of interstitial fluid separating the various channels are unimportant as long as the wall of the ascending limb of the loop of Henle, where solute transport is taking place, remains relatively impermeable to water, while the wall of the collecting duct permits the free movement of water.

Kuhn and Ramel (52) have worked out a mathematical treatment of the Kuhn model and studied the factors that would influence the concentration process. In the formula that they have derived:

$$\frac{C_x}{C_o} = e \operatorname{Exp} \frac{\lambda \cdot E \cdot x}{u \cdot a} \tag{29}$$

the concentration factor C_x/C_o is an exponential function of the conductivity of the membrane for the actively transported electrolytes (λ) of the e.m.f. driving their transport (E) and of the length of the counter-current system (x) and a root function of the linear velocity of flow (u) and of the cross-section of the fluid column (a). The term C_x/C_o represents the ratio of osmolarity at a distance x from the point of exit to that at the point of entry (C_o), and corresponds to U/P_{osm} in final urine.

Since the variation in the terms λ, E, u, and a in a biological system cannot be very great, it is understandable that concentrating ability is closely related to the length of Henle's loop (x) when different animals are compared (see Fig. 41).

Experimental Evidence for the Counter-Current System

The earliest experimental support for the operation of a counter-current system in the renal medulla was obtained by Wirz, Hargitay, and Kuhn in 1951 (113). They showed that there is a progressive increase in the osmolarity of tubular fluid from the outer medullary zone toward the papilla, where the value is similar to that of bladder urine (Fig. 43). Wirz (112) also showed by micropuncture that tubular fluid in the early distal tubule, i.e., at its exit from the ascending limb of Henle's loop, had an osmolarity that was less by some 100 mosm/l than that of fluid entering the loop which was isosmotic with plasma. The predictions from the Kuhn model were thus confirmed.

Of the many experimental findings published in support of the counter-current hypothesis, only some of the more important are mentioned here (33, 43, 100). The hyperosmotic environment at the tip of the papilla has

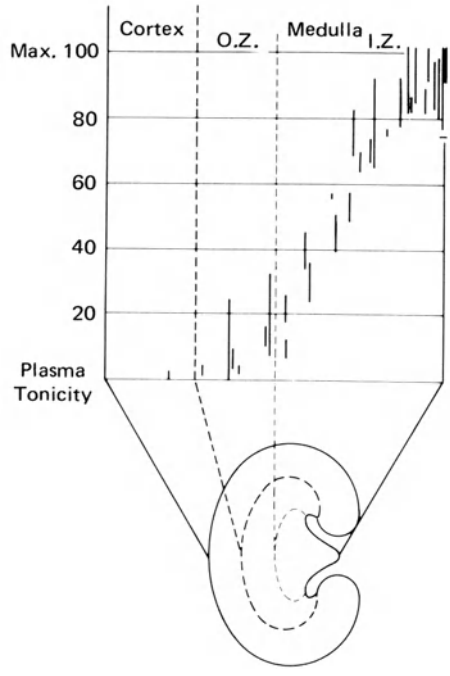


Fig. 43. Diagram showing the progressive increase in osmolarity of the tubular fluid from cortex to papilla based on measurements in the rat. The maximum osmolarity found in the papilla is taken as 100 percent, and that in glomerular filtrate (plasma), as zero. O.Z. = outer zone. I.Z. = inner zone of medulla. From Wirz et al. (113).

physiological consequences affecting structures constantly or transiently exposed to it. The erythrocytes in samples of blood taken by micropuncture from a papillary tip do, in fact, show the crenation typical of cells in a hypertonic suspending medium.

The notion that the driving force for the concentrating process is provided by active pumping of sodium in the ascending limbs of Henle's loops is confirmed by experiments on rats in which the renal papilla was exposed by resecting a portion of the cortex in order to render it accessible to micropuncture. Under these conditions it was found that in the inner medullary zone, remote from the apex of the loops of Henle, the osmolarity in the ascending thin segment was some 100 to 150 mosm/l less than in the descending limb at the same level.

Although, with the exception of the ascending limbs of the loop of Henle, the same osmotic pressure prevails in the collecting ducts as in the other structures at any given depth, there are differences in the overall

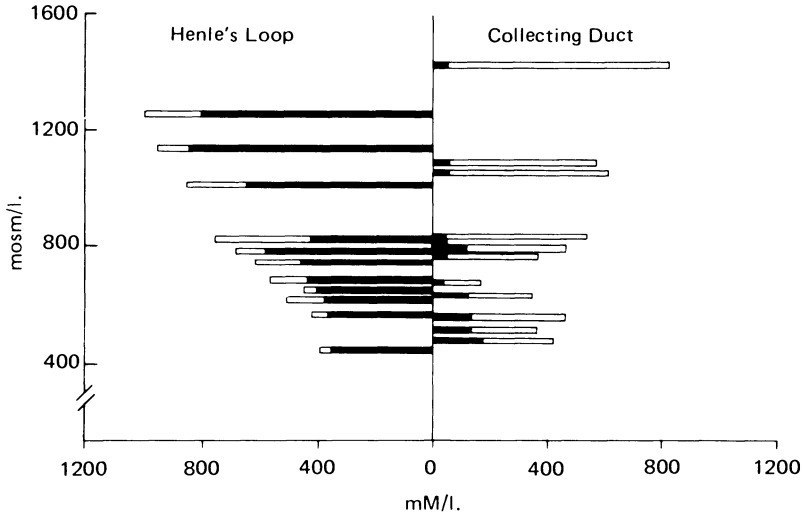


Fig. 44. The relationship between total osmolarity and the concentration of sodium chloride and urea in the fluid within Henle's loops and collecting ducts. NaCl is represented by solid bars, and urea by open bars. Twenty percent of the total osmolar concentration in Henle's loops is contributed by other osmotically active solutes, e.g., K^+ and creatinine, while in the collecting ducts the corresponding figure is 44 percent. In fluid samples of the same osmolarity taken from Henle's loop and from collecting ducts, the principal solute in the former case is sodium chloride while in the latter case it is urea. Modified from Gottschalk et al. (35).

chemical composition of the fluid in the different segments of the nephron. As may be seen from Fig. 44, the main contributors to the osmotic pressure in the loops of Henle are sodium and its attendant anions. In the collecting ducts the principal osmotically active solute is urea. There is, in fact, a sodium pump situated in the wall of the collecting duct which ensures that bladder urine is practically sodium-free if the state of sodium balance in the animal requires maximal conservation of this ion.

The Modus Operandi of the Counter-Current System in the Kidney

On the basis of the available data one may picture the counter-current system as operating in the following way. Sodium is pumped out of the ascending limb of the loop of Henle into the interstitium. Because of the low permeability of this part of the loop to water, it does not accompany the extruded sodium. Hence, the interstitial fluid, by virtue of its hypertonicity, can draw water out of other more permeable structures. These more permeable structures include the descending limb of the loop, the

collecting ducts, and the blood vessels. Thus, the osmotic pressure in all of these rises progressively as they approach the renal papilla in the same manner as in the Kuhn model.

So that the single effect and the counter-current stream of tubular fluid can operate together (as in Fig. 42), the sodium pump need sustain at each level only a limited concentration gradient of around 100 mM/l of NaCl. This is thermodynamically a very economical arrangement and enables the production of hypertonic urine to proceed at the expense of only a fraction of the energy that would be required to achieve the same result in a *single* step.

When it emerges from the ascending limb of Henle's loop into the distal convoluted tubule, the tubular fluid is hypotonic to plasma, and the difference between its content of NaCl and that of plasma is close to the limited concentration-difference arising from the single effect. The wall of the distal tubule is sufficiently permeable to water for osmotic equilibrium to be established by loss of water from the contents to its isotonic surroundings. This efflux of water, with some further reabsorption of NaCl, reduces the volume of the tubular fluid, so that only about one-half the fluid that leaves the loop of Henle ultimately reaches the collecting duct, in which it once again traverses the medulla. This residual fluid, during its passage toward the papilla, loses more water to its hypertonic surroundings. In addition, some further sodium reabsorption takes place in the collecting ducts and is accompanied by a passive flux of water; this contributes further to the reduction of fluid volume, so that the final urine amounts to only about 1 percent of the GFR and its osmolarity is very nearly as high as that of the interstitial fluid at the papillary tip.

Role of Urea in the Concentrating Process

The role of urea in the concentrating process has long remained obscure. In various pathological and experimental situations it has been shown that only when a sufficient filtered load of urea is present can urine of maximum osmolarity be formed.

It was therefore believed that urea must play an active part in the concentrating mechanism. More detailed investigations revealed, however, that this was not true in the sense that urea assists in the concentration of other substances. As may be seen from Fig. 45, the concentration of other solutes goes down rather than up when the concentration of urea is increased.

The initiation and continuation of the concentrating process depend primarily upon the sodium pump in the counter-current multiplier, assisted by the varying water permeability of the different segments of the

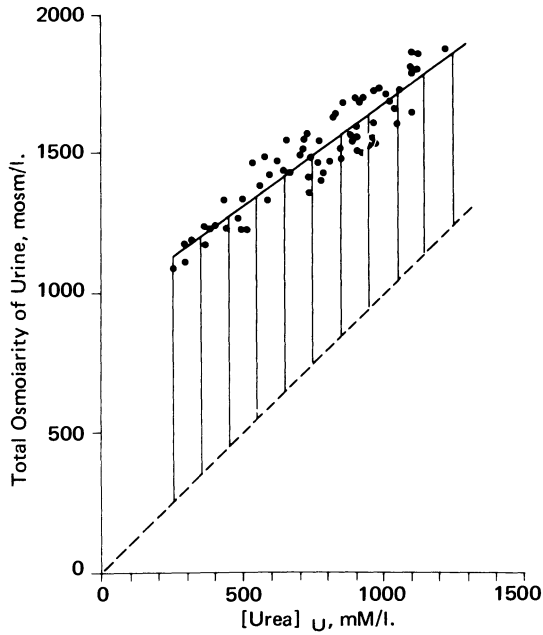


Fig. 45. The relationship between total osmolality of the urine and urea concentration in the dog. The intervals between the dashed line and the solid lines indicate the contribution of solutes other than urea. After Reinhardt et al. (77)

nephron, this permeability being partly under the control of the antidiuretic hormone, ADH (see page 87). Urea on the other hand becomes concentrated passively by removal of water and by counter-current diffusion without playing any active part in the concentrating mechanism, and so it cannot increase the concentration of other substances. It does, however, contribute to the attainment of a high total osmolarity, and since urea is a metabolic end product that is of necessity excreted in the urine, it is important to the conservation of water that urinary urea be in high concentration.

Significance of the Medullary Blood Flow in Relation to Concentrating Ability

The scheme shown in Fig. 46, based on micropuncture experiments, illustrates what proportions of the glomerular filtrate are reabsorbed in the cortex and the medulla, respectively. In this greatly simplified diagram the difference in perfusion between the cortical and juxta-medullary nephrons (see page 2) is not taken into account. It appears that about 30 percent of

the filtered volume of fluid is reabsorbed in the medulla and so must be carried away by the medullary circulation. The blood flow required for this purpose, however, could have a dissipating effect on the medullary osmotic gradients because osmotically active solutes could be carried away at the same time. Such an effect is, however, minimized; first, because the medullary blood flow is small compared to that of the cortex, and secondly because the close apposition of the descending and ascending limbs of the vasa recta favors a counter-current diffusion or exchange (see below) of osmotically active solute from ascending to descending limbs.

The influence of medullary blood flow upon the concentrating ability of the kidneys can be most readily demonstrated by raising this blood flow selectively. This can be done by raising the arterial blood pressure which, because of auto-regulation, produces very little change in the cortical blood flow or in the GFR but leads to a rise in medullary blood flow. Under these conditions there occurs a fall in urinary osmolarity, even though the GFR and hence the inflow into Henle's loop, is virtually constant (Fig. 47).

Whenever the medullary blood flow exceeds the optimum, osmotically active solutes are washed away and the build-up of concentration gradients is to a greater or lesser extent impaired. Such imbalance can be induced by the administration of drugs such as theophylin or papaverin, which dilate the medullary vessels; or it may occur in the condition of "shock," where filtration almost ceases while the medullary circulation continues. However, an increase in medullary blood flow is also invoked under physiologi-

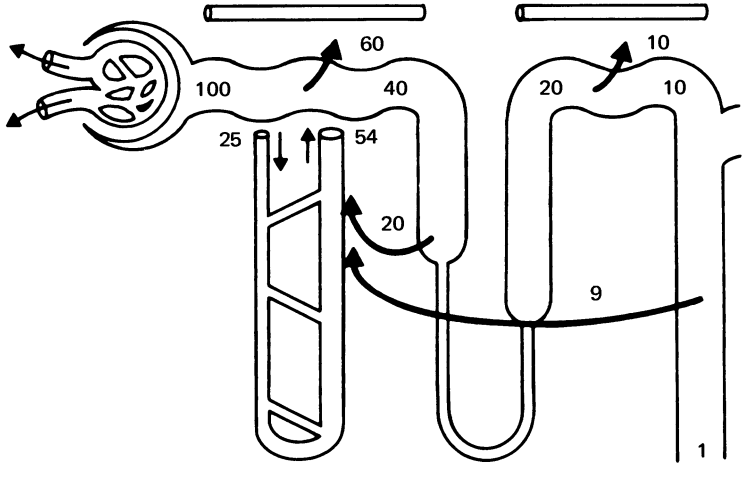


Fig. 46. The fluid-balance of the kidney assuming a GFR of 100 ml/min and a medullary blood flow of 25 ml/min. Net water movement is indicated by heavy arrows. The numbers refer to millilitres per minute for the whole kidney.

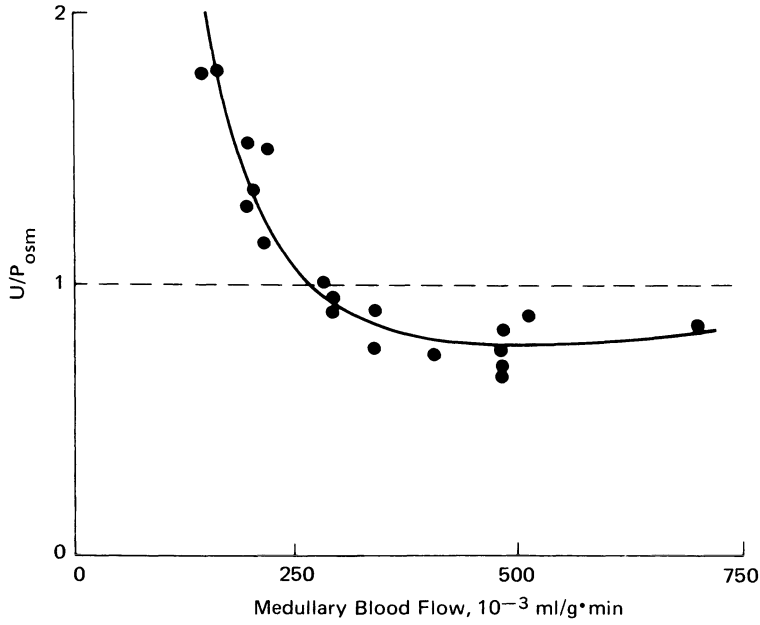


Fig. 47. The relationship between medullary blood flow and urinary osmolarity in the dog. Blood flow was altered by changing the perfusing pressure in the renal artery. From Thurau and Deetjen (95).

cal conditions in order to reduce the build-up of concentration gradients as, for example, during the onset of a water diuresis when medullary blood flow is increased (97).

Counter-Current Diffusion

The arrangement of the vasa recta in the form of bundles in which the ascending and descending vessels are in close apposition to each other favors the build-up and maintenance of osmotic gradients at the tip of the papilla by the process of counter-current diffusion.

The principle of counter-current diffusion (or "exchange") is more easily understood by considering the case of heat transfer rather than osmotic gradients (see Fig. 48). Suppose that 10 ml/min of water at a temperature of 30°C flows into tube (a) and passes through a heating device which delivers 100 cal/min to the water, thereby raising its temperature to 40°C. Suppose then that the tube is bent upon itself as in Fig. 48(b). Provided that there is no heat lost to the surroundings and heat

transfer can take place freely between the two limbs of the tube, the temperature at the point of exit will rise to 40°C, just as in arrangement (a). However, within the closed system the water reaches a higher temperature in (b) because of heat exchange between the ascending and descending limbs, which delivers water at a higher temperature to the heat source.

In the loops of the vasa recta in the renal medulla it is the concentration of osmotically active particles such as Na^+, Cl, and urea, rather than the temperature, that is influenced by the counter-current effect, and in this way a high osmolarity can be maintained at the apices of the loops.

Gases dissolved in blood can also undergo counter-current diffusion, and this could explain the high P_{CO_2} of urine, since the CO_2 produced from metabolism in the papilla maintains a high P_{CO_2} at the tip by counter-current diffusion. Since CO_2 is freely diffusible, the fluid in the collecting ducts equilibrates with the high P_{CO_2} of the tissue (41).

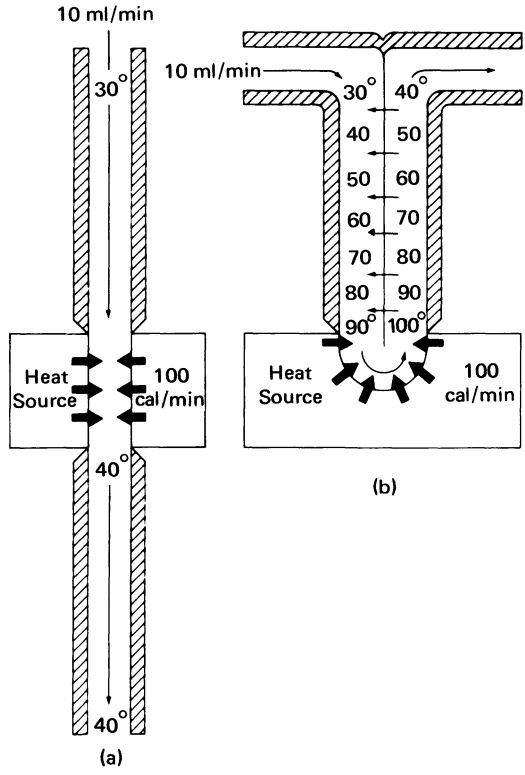


Fig. 48. The principle of countercurrent diffusion demonstrated in a heat-exchanging system with constant input of heat. For description see text. [Modified from Berliner et al. (5)]

As far as substances utilized by the medullary cells for their metabolism are concerned, the counter-current mechanism has the opposite effect. The most fully investigated substance of this type is oxygen (49). As a result of the low but still significant uptake of oxygen in the papillary region, blood that leaves the papilla in the ascending vasa recta has a very low oxygen content but is in intimate contact with the near-arterial blood in the descending vessels. Since oxygen is freely diffusible, it tends to be short-circuited from the descending to the ascending blood stream, and so the delivery of oxygen to the papillary tip is diminished. The oxygen tension in this region is very low, and fluid emerging from the collecting ducts has equilibrated with the surrounding tissue and also has a low oxygen tension. The observation of Planer made in 1859 (74) that urine is practically oxygen-free was confirmed by many subsequent reports but could not be satisfactorily explained before the advent of the counter-current theory.

It should be mentioned that the renal medulla is well adapted to the low oxygen tension to which it is exposed. Its metabolic rate is considerably lower than that of the cortex (see page 30), and it makes extensive use of anaerobic reactions. Enzymes concerned with glycolysis and anaerobic energy-yielding reactions are prominent in medullary tissue, and in contrast to the cortex (see page 58) the medulla relies in large measure on glucose as a metabolic substrate. Further evidence of anaerobic activity is found in the concentration of lactic acid in blood leaving the medulla, which is actually higher in the vasa recta than in arterial blood (100).

Significance of the Urine-Concentrating Mechanism

The normal nutritional state of man requires that he excrete each day about 1200 mosm of "obligatory" urinary constituents, which include metabolic end products such as urea and creatinine and also certain ions, of which an excess is often ingested in the diet. This is a considerable quantity compared with the total osmotically active material in the body. A normal individual of 70 kg body weight has a body-water content of about 42 liters, of which 14 liters are extracellular (ECF) and 28 liters are intracellular (ICF). The osmolarity of both ECF and ICF is about 300 mosm/l., which means that the total osmolar content of the body is 42 $\times$ 300 = 12,600 mosm. The daily osmolar load requiring excretion is therefore about 10 percent of the total body content and about 30 percent of the extracellular content. If this load were excreted in a solution isosmotic with plasma, the volume of urine required would be about 4 liters. Thus, the ability of the human kidney to produce urine with four times the osmolarity of plasma means that the urinary water can be reduced below 1

liter per day and under conditions of water deprivation the chances of survival are correspondingly improved (see page 129).

Even when there is no shortage of water, human urine generally has an osmolarity equal to three times that of plasma, so that the daily output tends on the average to be about 1.5 liters, which is more conveniently voided than would be a volume of 4 liters. The desert animals for which data are shown in Fig. 40 have become independent of any source of drinking water by virtue of their highly developed urinary concentrating mechanisms. By increasing the urinary osmolarity by a factor of 17 as compared with the plasma, these animals can excrete their obligatory solute load in the water derived from the oxidation of foodstuff.

Water Diuresis and the Effect of Antidiuretic Hormone (ADH)

When the body is hydrated, the kidney is capable of excreting a urine one-sixth the concentration of the blood plasma, i.e., with an osmolarity of 50 mosm/l.

As has already been mentioned, fluid emerging from the ascending limb of Henle's loops is "hyposmotic" with respect to plasma (see page 78). In the case of the concentrating kidney, i.e., during antidiuresis, this hyposmolarity as the fluid passes through the distal convoluted tubule is changed to isosmolarity and in the collecting ducts becomes, as a result of osmotic equilibration, hyperosmotic. Under these conditions the water permeability of the distal convolutions and the collecting ducts is kept high by the action of ADH. Only in the presence of ADH is water permeability of the distal portion of the nephron sufficiently great for osmotic equilibration to take place. In the absence of ADH the fluid delivered from the loops of Henle remains hyposomotic throughout its passage to the end of the collecting duct.

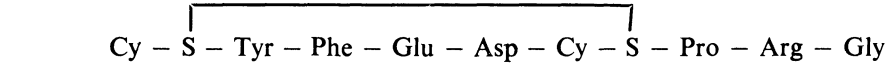
In the production of water diuresis the withdrawal of the effect of ADH is reinforced by an increase in medullary blood flow (see page 82), which favors the wash-out of osmotically active particles from the medulla; the osmolarity of the medullary interstitium, as well as the fluid in the hairpin bends of the loops of Henle and of the vasa recta, approaches the osmolarity of plasma. A further consequence is lessened withdrawal of water from the tubular fluid and hence an increased volume flow through the distal nephron, allowing less time for osmotic equilibration to be reached with the surroundings. It has been shown, in fact, that even if the ADH activity of the blood is unaltered, a hypotonic urine can still be produced if the rate of flow through the distal tubule is sufficiently accelerated (95),

ADH exerts its effects on water permeability only in the distal convolutions and the collecting ducts. The proximal tubules (see page 31).

which have a high water permeability under normal circumstances, are unaffected by the administration of ADH, and even complete withdrawal of ADH does not lead to a measurable reduction in their water permeability (103). In the ascending limb of the loop of Henle the very low water permeability of the luminal membrane is unaffected by ADH. (The effect of glucocorticoids in reducing the water permeability of the distal nephron and so antagonizing the action of ADH, was referred to on page 34.)

In experiments on rats, in which endogenous release of ADH can be almost completely suppressed either by full hydration or by alcohol-narcosis, an interesting dose-response relationship was observed with respect to exogenous hormone (26). With increasingly smaller doses there is an almost linear increase in water reabsorption, but as little as 1 to 1.5 μU of ADH is sufficient to produce a maximum response, so that further increases in the dosage are without effect. Superfluous ADH is then excreted by the kidney very rapidly, the half-time of the process being about five minutes. Only amounts of ADH less than the dose required for maximum response appear to be bound by the receptor sites on the membranes of tubular cells, where the hormone affects water reabsorption for about 50 minutes before being inactivated. The volume of water reabsorbed in response to ADH given in the dose at which the dose-response curve flattens out is surprisingly reproducible in rat experiments, and amounts to about 4 ml, the so-called ADH-equivalent. Since the kidneys of a rat weigh about 1.5 g, whereas the human kidneys weigh about 300 g, it might be expected that in man the ADH-equivalent would be 200 times greater. Thus, other things being equal, it would appear that in man around 26 liters of water per day are reabsorbed under the influence of ADH. Absence of ADH is encountered in the pathological condition of diabetes insipidus, and in fact the actual amounts of urine passed correspond reasonably well with the result of the above calculation. This agreement could be pure coincidence but is sufficiently striking to encourage some extrapolation from rat data to the human subject.

The chemical structure of ADH has been established and its synthesis accomplished (21). It is an octapeptide, and in man and the majority of mammals the amino acid sequence is as follows:



As regards the mode of action of ADH, information is still incomplete. On the one hand, it is held that the disulphide bridge in the hormone enters into combination with a free sulphydryl group located in a receptor on the cell membrane and causes a change in the configuration of the membrane

structure resulting in an increase in the diameter of pores situated in it. Other authors prefer to postulate a more indirect action, whereby ADH promotes the liberation of hyaluronidase, which in turn degrades some of the mucopolysaccharide found in the intercellular cement. The increased water permeability, with this hypothesis, would then result from a loosening of the intercellular connections. Most recently it has been proposed that ADH promotes the conversion of ATP to cyclic 3′, 5′ AMP (adenosine-3′-5′-phosphate), which as the "second messenger" has been implicated in other hormonal actions (66). This theory is supported by the observation that cyclic AMP does mimic the action of ADH on the amphibian bladder and isolated collecting duct.

Evolution of the Kidney

Homer Smith in his book *From Fish to Philosopher*, in which he traces the evolution of salt and water regulatory systems, writes of the homoeothermic kidney, "What engineer, wishing to regulate the composition of the internal environment of the body, on which the function of every bone, gland, muscle and nerve depends, would devise a scheme that operated by throwing the whole thing out sixteen times a day—and rely on grabbing from it as it fell to earth—only those precious elements which it wanted to keep?" In fact, if it were simply a question of excreting through the kidneys each day a total of 1200 mosmols of metabolic waste products, predominantly urea, the filtration-reabsorption design of the mammalian kidney would make it a most inefficient instrument. However, the complexity and variety of the renal mechanisms that maintain the constancy of Claude Bernard's "milieu intérieur" can be explained on evolutionary grounds as representing adaptation to changing external environments (46).

Life arose in the oceans where unicellular organisms had minimal regulatory problems. Nutrient substances were taken up by simple diffusion from the virtually inexhaustible reserves present in the oceans; metabolic end products diffused out of the cell into a virtually limitless sink. Concentration differences between a cell and its surroundings can be maintained by metabolism, and the dimensions of a unicellular organism are such that the required movements of material can take place by diffusion within a few milliseconds. The following equation defines the path length (x) along which a particle can diffuse in a particular direction in time t:

$$x = \sqrt{D \cdot t} \tag{30}$$

The diffusion coefficient D for particles of low molecular weight in aqueous solution under favorable conditions is 10^{-5} cm$^2 \cdot$ sec^{-1}. If one

considers a hypothetical spherical organism with a radius of 1 cm, the time required for a particle to diffuse from the periphery to the center would be more than a day. For the development of multicellular organisms it was therefore essential for the cells in the interior of the organism to be surrounded with an extracellular fluid and to evolve an organ that would ensure the constant composition of the extracellular fluid. It was also necessary to evolve some form of circulatory system to provide communication between the extracellular fluid and the organ responsible for controlling its composition.

The origin of the vertebrates has been placed in the Cambrian Era, when geological perturbations probably resulted in representatives of the animal kingdom being cut off from the sea. In the fresh water of lakes and rivers only those forms of life could survive which succeeded in protecting themselves against an inflow of water from their new hypotonic environment.

This objective was achieved in the most primitive freshwater fish (Ostracodermia) by the development of a water-tight armor. This in turn had the disadvantage that it restricted the free movement of the wearer. Thus, the process of natural selection led to the survival of animals with the ability to excrete superfluous water.

The excretion of this surplus water by active transport would be very costly in terms of energy expenditure. As a result of the first unicellular organisms coming into being in the prehistoric seas, the osmotic pressure of these organisms was determined by the salinity of their external environment, and when their multicellular descendants appeared, these developed an extracellular "milieu intérieur" of the same tonicity as the prehistoric seas. This sequence of events provides an explanation of the widespread occurrence in the animal kingdom of extracellular fluid with an osmolarity of 300 mosm/l. In such a solution 300 mosm of solute are dissolved in 185 moles of water. From a thermodynamic point of view the most favorable method of effecting the movement of water is to make the primary event an active transport of sodium (which is the predominant cation in the extracellular space). Anions such as chloride and bicarbonate will then follow secondarily to maintain electrical neutrality, and water movement will occur as a tertiary event brought about by osmotic forces.

Active transport of sodium would not, however, provide by itself a solution to the problem of water elimination, since for an animal living in fresh water the loss of sodium would be intolerable. So it turns out that filtration, followed by reabsorption of solute, which at first sight seems a nonsensical arrangement, is in fact a most satisfactory method of regulating water turnover. The energy necessary for filtration is provided by the beating of the heart, and the filtrate during its passage through the tubular segments of the nephron is modified in composition by the active transport

of useful substances like electrolytes, glucose, and amino acids back into the extracellular space, while the residual water and unwanted metabolites are released into the environment.

The combination of filtration with reabsorption is a characteristic of kidneys in a wide variety of animals ranging from very primitive vertebrates to homo sapiens (see Fig. 49). A few creatures, which have returned to a marine habitat, have dispensed with the filtration process to a greater or lesser extent, so that certain fish are totally aglomerular.

A further problem in regulation of the internal environment made its appearance when vertebrates began to colonize the land. The first step in adaptation to terrestrial life can still be observed in amphibians. These return periodically to the water in order to make good the fluid losses incurred while on land. Evaporative water loss occurs through the skin, but water uptake can also take place by this route. By means of a nervously controlled "throttle" in the preglomerular circulation and by the development of a hormone (ADH) capable of enhancing water permeability, these animals were able to prolong the time that they could spend on land.

Reptiles and birds conserve their body-water by means of an outer integument that has a very low permeability to water. They have reduced their reliance on filtration and excrete uric acid instead of urea as an end product of protein metabolism. The nitrogen content per mole of uric acid is twice that of urea. Since uric acid is excreted in crystalline form via the cloaca, the water requirement of these animals is rather small.

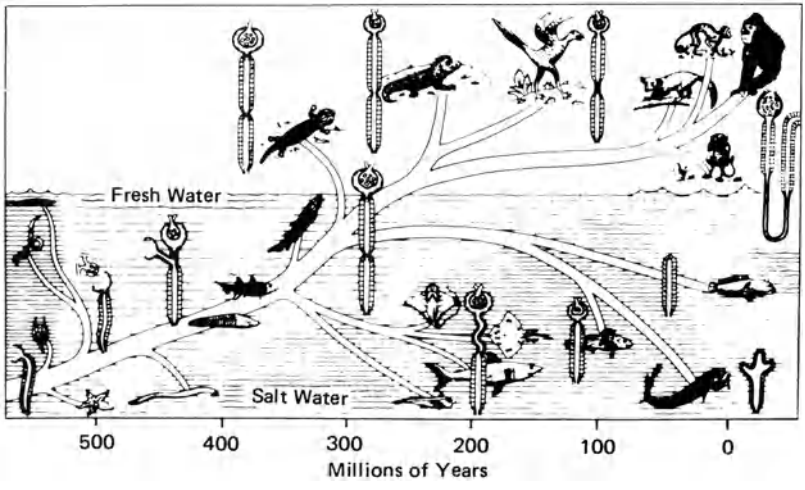


Fig. 49. Diagram of the development of the nephron and its adaption to environmental conditions during the course of evolution. [Modified from H. W. Smith (87).]

One of the most striking characteristics of mammals is their temperature-regulating system that protects them from the effects of fluctuations in the temperature of their surroundings. The heat production from metabolism which this system demands leads to an increased oxygen uptake, so that the land, where oxygen is plentiful, is well suited to their needs. Mammals have retained the same method of disposing of nitrogenous waste, i.e., in the form of urea, as is employed in the fishes and amphibia, and they have not lost the ability to eliminate excess water through the kidneys. The important new factor found in the mammals is the development of a concentrating mechanism that makes possible a satisfactory adaptation to terrestrial conditions (see page 86), especially those found in arid regions of the world, the denizens of which can survive without the necessity to drink at all.

Man, whose urine may be four times more concentrated than his plasma, does not need to take in fluid more than once or twice each day. This urinary hypertonicity, although it is relatively trivial compared to that attainable by desert mammals, has contributed a great deal toward extending man's versatility, as regards both his habits and his chances of survival. How great this contribution is may be appreciated from a consideration of the clinical picture of diabetes insipidus, in which either the secretion of ADH is impaired or the kidney suffers from an inborn insensitivity to ADH (see page 87). Patients suffering from this disorder may need to drink as much as 25 liters of fluid per day and thirst may interrupt their sleep several times each night.

A consideration of the steps by which these renal functions have evolved helps toward an understanding of the complexity of the mammalian kidney. The intricate coordination of the functions of the different segments of the nephron is directed toward maintaining the volume and composition of the ECF nearly constant. This means that all constituents that may be present in excess must be removed, whether the excess arises as a result of metabolic reactions or a surplus in the diet. This objective of maintaining the constancy of the milieu intérieur is accomplished through the continuous passage of a fraction of the extracellular fluid (about 1 percent per minute) through the glomerular filter. Substances that, because of their low molecular weight, traverse this filter but at the same time are valuable to the body are reabsorbed in the proximal tubule, and the considerable length of this segment provides a good safety margin in relation to this salvage operation. At the end of the proximal convolutions there remains a fluid practically devoid of glucose, amino acids, and bicarbonate, while substances to be excreted, such as urea and hydrogen ions, have already undergone some increase in concentration. This modified filtrate then enters the distal nephron where, under the controlling influence of aldosterone and ADH and depending on the state of balance existing at the time, more or

less of the various electrolytes and solvent water are returned to the body. Any surplus of electrolytes and water not required to ensure the constancy of the volume and the composition of the extracellular fluid is then excreted along with the waste products of metabolism.

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SALT AND WATER BALANCE

Water as the Basis of Life

In warm-blooded animals water makes up about 60 percent of the body weight and exists as a solution of organic and mineral substances. This water is in constant exchange with the environment as a result of periodic uptake from the gut and continual loss through the skin, respiratory passages, and kidney. Within the body the water is distributed in several more or less discrete compartments whose contents are called the "body fluids." The anatomical boundaries separating these compartments and the differences in the solutes present in each are of fundamental biological significance.

The unusual physical and chemical properties of water confer upon it a unique capability to support life processes, and these unusual properties derive from its dipole structure and the readiness with which it can form hydrogen bonds. The oxygen atom has a greater affinity for electrons than the hydrogen atom, so that as shown in Fig. 50, the oxygen position is

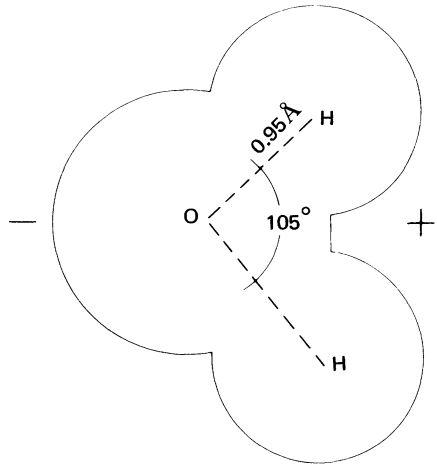

Fig. 50. The water molecule as a dipole.

Table 6. Some physical properties of water compared with those of other common solvents. (Data refer to 20°C unless otherwise stated.)

Substance	Specific heat (cal/g)	Latest heat of vaporization (cal/g)	Surface[a] tension (dynes/cm)	Dielectric constant	Viscosity (millipoises)
Water	0.999	585	73	80	10.1
Ethanol	0.582	204 (78°C)	22	—	11.9
Ethylene glycol	0.571	191 (197°C)	47	41	173.0
Diethyl-ether	0.547	84 (35°C)	17	4	2.5
Acetone	0.528	132	24	21	3.3
Acetic acid	0.468	97 (118°C)	28	7	12.1
Chloroform	0.234	61 (40°C)	27	5	5.6

[a] At interface with air.

electro-negative relative to hydrogen in the water molecule. Studies of infra-red and micro-wave spectra have revealed that the bond angle between the two hydrogens is 104.45°. The covalent O-H links are very stable over the temperature range of living systems, while the hydrogen bonds that attach each water molecule to four neighbouring molecules are relatively weak, although it must be added that the precise molecular geometry of water is as yet undefined (45a).

In Table 6 a number of the interesting physical properties of water are listed and compared with those of other common liquids.

Solvent Properties of Water

Water is a versatile solvent; it is only in aqueous solution that substances can pass into and out of living cells, and within cells, only substances in solution can react with one another. Hence by virtue of its great solvent power water provides a more favorable medium for life processes than would any other liquid. It is equally significant that water is itself relatively inert chemically, so that the substances dissolved in it retain their chemical properties unaltered.

The very high surface tension of water is another useful property attributable to its ability to form hydrogen bonds. This characteristic is the basis of the so-called capillarity which contributes to the hemodynamic features of flow in small blood vessels.

Thermal Properties of Water

The high specific heat of water not only reduces the fluctuations of temperature on the Earth's surface but also makes possible the maintenance of a stable body temperature in living animals. This property of storing and releasing heat more slowly than other solvents protects the organism from major variations in temperature as a result of heat loss or heat generation.

A 70-kg man produces under conditions of moderate activity about 2800 Kcal/day, an amount of heat sufficient to raise the temperature of 1 liter of water by 2800°C. This means that if heat loss were prevented, the temperature of the body, since it is largely made up of water, would rise by about 40°C. Because of the high specific heat of water as compared with other solvents, this is the smallest possible temperature rise that could result from the delivery of this quantity of heat. Under normal environmental conditions the bodily mechanisms can readily dissipate such a quantity of heat, so that internal temperature would remain relatively constant. If, however, water exhibited the specific heat of, say, alcohol, the equivalent rises in temperature could be twice as great, and if it behaved like chloroform, nearly four times as great. The regulatory mechanisms would then be called upon to prevent a rise in body temperature of 80 to 160°C, a task made unnecessary by the thermal properties of water.

The heat of vaporization of water is the highest possible, and this fact permits the existence of life practically everywhere on the Earth's surface. If environmental temperature becomes equal to or greater than that of the body, then heat loss can occur only by the evaporation of surface water, and its high heat of vaporization permits maximum dissipation of heat for each unit volume of sweat evaporated.

Neutrality and Ionization Constant

Pure water contains H^+ and OH^- ions in equal concentrations and is therefore neutral. Its dielectric constant is very large and this minimizes the mutual attraction between ions of opposite sign; ionizable substances therefore dissociate most readily in water, and so proteins like serum albumin that contain many charged groups are freely soluble in water. The ability of water to facilitate dissociation is very important in physiology because many of the basic processes of life, such as the generation of membrane potentials and the conduction of nerve impulses, can take place only in the presence of ions.

The near-neutral reaction of water is ensured in living systems by the buffering effects of carbonic acid and its salts. Carbon dioxide, which is

ubiquitous, dissolve very easily in water, and when hydrated it can, even in traces, enhance the solvent power of water. It is therefore understandable that the solid minerals of the Earth's crust are gradually washed into the oceans. The CO_2-bicarbonate buffer system determined the slightly alkaline reaction of the primitive oceans, and is reflected today in the slightly alkaline reaction of protoplasm.

It is known that the permeability of biological membranes to different ions is not well correlated with the diameter of the ion. Sometimes ions of relatively large diameter can pass more readily through a membrane than can smaller ones, and this paradoxical behavior has been accounted for by their varying degrees of hydration. Since the strength of the electric field at the surface of an ion is inversely proportional to the diameter of the ion, it was inferred that smaller ions exert a greater attractive force on the water dipoles. More recently the greater hindrance to the movement of small ions has been attributed to structural changes in the water itself which result from the presence of these ions. Thus, the presence of a particular ion in the

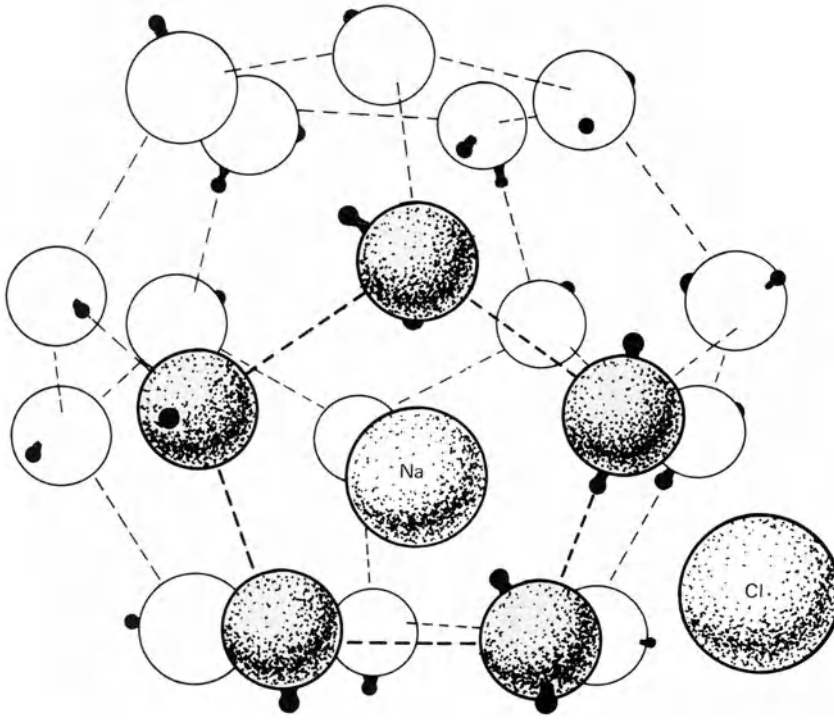

Fig. 51. The possible location of the sodium ion in relation to its hydration shell.

structural lattice of water causes a change in the lattice, rendering it more or less dense and so influencing the movement of the ion accordingly (Fig. 51).

Input and Output of Water

Thirst, or the urge to drink, regulates very effectively the uptake of water. The sensation of thirst is probably a consequence of cellular dehydration detected by osmoreceptors. These receptors lie in the supraoptic and paraventricular nuclei and are likely to be identical with the neurones responsible for the secretion of ADH (59). They are supplied by deep branches of the internal carotid artery, and their adequate stimulus is a change in the osmotic pressure of the blood. Thus, the injection of hypertonic saline into the carotid artery leads to an activation of these neurones (15) and an inhibition of water diuresis. Stimulation or inhibition of ADH production can also be brought about by volume receptors in the low-pressure areas of the circulatory system or through nonspecific activation of the hypothalamus; for example, by emotion. It is a matter of common experience that the feeling of thirst can be assuaged by the drinking of water. Adolph (1, 51) has found that the quantity of water drunk by a thirsty dog is almost exactly the amount required to restore body water to normal, i.e., to make good the water deficit. This is so whether the water actually reaches the stomach or is discharged through an esophageal fistula, and since drinking is normally completed before the imbibed water is absorbed into the blood stream, it is clear that the volume of water drunk must in some way be monitored during the act of drinking.

While the loss of water from the body is a continuous process, its replacement by drinking takes place only intermittently and matches the water deficit with differing degrees of accuracy in different animals. Adolph (1) has observed that the smallest water deficit to which a dog will respond represents about 0.5 percent of the body weight. It is clear, however, that the actual extent of water deficit required to provoke drinking depends on concomitant changes in the salt content of the body.

In contrast to the dog or cat, man will normally not restore moderate water deficit (i.e., 2 to 3 percent of the body weight) completely when the opportunity to drink presents itself. Bodily exertion appears to suppress the sensation of thirst so that "voluntary" dehydration of up to 4 or 5 percent of the body weight is tolerated. Toward the end of the period of exertion, and immediately thereafter, enough water is taken in to replace some 75 percent of deficit. The remaining loss is made good somewhat later—commonly with the evening meal. It seems likely that the simultaneous loss of salt in the sweat is partly responsible for the tendency in man not to make good immediately and precisely the water deficit incurred

during exertion in a hot environment. Actually, if salt is administered at hourly intervals during exertion, water intake is more accurately matched to need by the operation of the thirst mechanism. Presumably the sensation of thirst that arises immediately following an injection of hypertonic saline is brought about by cellular dehydration. The locus of action of this stimulus in the central nervous system was established by Anderson (3), who found in experiments on goats that the micro-injection of hypertonic solutions of sodium chloride into the medial hypothalmus near the wall of the third ventricle caused drinking in these animals. The most striking effects were obtained when the injection was made in the vicinity of the paraventricular nucleus. Injections of isotonic or hypotonic solutions were without effect. Hypertonic fluids were equally ineffective if injected into other areas of the brain. Further studies of the "drinking center," using implanted platinum electrodes, have revealed that the drinking response can be modified by other stimuli applied to its surroundings. Local cooling or heating of the supra-optic area and rostral hypothalamus were found to induce rejection of water by the dehydrated animal or drinking by those previously satiated. This finding indicates that the thermoregulatory mechanism can also influence water turnover (4).

Daily water balance in man is best illustrated by an example. Consider, for instance, a 70-kg man whose body surface is 1.72 m^2 and whose diet provides adequately for his caloric requirements. Suppose that this diet consists of 100 g protein, 300 g carbohydrate, and 100 g fat, and that the intake of sodium chloride is 156 meq per day. The total weight of such a diet will be about 1.25 kg, because of its water content.

Water is present in food either as "preformed" water (which can be measured by drying the food to constant weight in an oven) and also "metabolic" water formed during oxidation of the food in the body. From a knowledge of the constituents of the diet, its content of preformed water can be calculated assuming that about 60 percent by weight is water and in the case of the diet mentioned in our example it would amount to about 750 ml (see Table 7).

Also from a knowledge of the dietary constituents the yield of metabolic water may be calculated. Carbohydrates, for example, will be completely oxidized according to the equation:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

which, taking into account molecular weights, can be written

$$180\text{ g } C_6H_{12}O_6 + 192\text{ g } O_2 \rightarrow 264\text{ g } CO_2 + 180\text{ g } H_2O$$

from which it follows that 1 g of carbohydrate gives rise to 0.6 g of water.

Table 7. Components of the water-balance in a 70-kg man on a daily diet containing 300 g carbohydrate, 100 g fat, 100 g protein, and 156 meq NaCl.

Intake (ml/24 hr)			Output (ml/24 hr)		
Source	Obligatory	Facultative	Route	Obligatory	Facultative
Preformed water in food	750	—	Skin and lungs	840	
Water of oxidation	320	—	Urine	760	> 1000
Drinking	630	> 1000	Feces	100	
Minimum	1700	—		1700	—
Total	2700			2700	

Hence, since 0.6 g of water corresponds to a volume of 0.6 ml, the 300 g of carbohydrate will produce 180 ml of metabolic water.

Protein, when degraded to urea, produces 0.4 ml of water per gram. Fat, because of its high content of hydrogen, produces 1 ml of water for each gram oxidized.

In our example (see Table 7) the calculated value for total metabolic water is 320 ml, as compared with 750 ml of preformed water, so that in all 1070 ml of water is made available to the body along with the food. The other method of taking in water, *viz.*, by drinking, is more affected by the habits and social customs of the individual. There is, however, a minimal requirement that must be satisfied in order to maintain water balance. This minimum requirement can be estimated by taking account of the so-called obligatory water losses.

The term "insensible perspiration" is used to describe the loss of water by diffusion through the skin, exclusive of sweat, together with the loss from respiratory passages resulting from the fact that expired air is saturated with water vapor. These water losses through the skin and air passages can be determined quite accurately from changes in body weight. They amount to about 0.5 ml/hr/kg body weight, and accordingly a figure of 840 ml/day is inserted into the balance sheet for our 70-kg subject (see Table 7).

One can calculate the minimum volume of urine required to excrete the waste products of protein metabolism and any superfluous electrolytes. So long as the subject is not salt-deficient, the 156 meq of sodium chloride ingested with his diet will be quantitatively excreted and so will contribute with its equivalent anions 312 mosmol to the "osmotic load" of substances presented to the kidneys. Normally the diet will also contain some 50

meq of potassium which, together with accompanying anions, will contribute a further 100 mosmols.

100 g of dietary protein contains some 16 g of nitrogen, of which 90 percent (i.e., about 15 g) is excreted in the urine, and of this, some 90 percent is in the form of urea. In our example 14 g urea nitrogen must be excreted per day, and from the chemical formula of urea with its two nitrogen atoms in a molecular weight of 60 corresponds to 30 g of urea or 0.5 osmo (i.e., 500 mosm).

The total load of osmotically active substances requiring excretion is therefore 912 mosm (312 NaCl + 100 KX + 500 urea). Urinary concentration dictates the volume necessary to contain this load and a considerable range of concentrations is possible within the extremes of 50 mosm/l. during water diuresis and 1200 mosm/l. during maximum water conservation. Thus, if it is necessary to conserve water, it is important to excrete only the minimum volume of urine, and at the maximum attainable osmolarity 912 mosm of solute would require $912 \div 1200 = 0.76$ liter of solvent water. In addition to the urinary volume the feces contain about 100 ml of water, so that the total unavoidable water loss amounts to some 1700 ml/day, being the sum of the losses via the respiratory tract, skin, kidneys, and gut.

The difference between this obligatory water loss and water derived from food ($1700 - 1070 = 630$ ml) gives a measure of the intake of water necessary to remain in water balance. Social customs and dietary habits affect both salt intake and the consumption of beverages so that ingestion of fluid tends to exceed the minimum requirement by a liter or more a day. This excess, provided it is not used up in evaporative cooling, as may happen in hot environments, is excreted by the kidneys and so raises the urinary volume above the minimum figure. Since this excess water is not accompanied by solute (52), it causes a fall in urinary osmolarity, commonly to about 600 mosm/l. In situations where evaporative loss is increased, the minimum water intake required for balance can be defined as that required to provide the minimum urine volume without weight loss. When heavy exercise is carried out in a hot environment this may be as much as an additional 20 liters a day.

Water Absorption from the Gut

In response to thirst, water is drunk and reaches the digestive tract, where by far the greatest proportion of it is absorbed in the small intestine, the mucosal surface of which is very extensive. Only about 35 percent of the water drunk reaches the large intestine, where a further fraction is reabsorbed during the conversion of the fluid chyme into semi-solid feces.

In the digestive tract, ions are added to the imbibed water, and this process in fact begins in the stomach where the secretion of HCl leads to acidification of the contents. At the same time, water passes into the interstitial spaces in the wall of the gastro-intestinal tract and by osmosis into the blood. This movement of water is sometimes called "extrinsic osmosis" or "classical osmosis" (18, 57). It is presumed to take place passively. It probably accounts for only a small part of water absorption from the intestine, because the measured water permeability of the intestinal lining (i.e., its osmotic permeability) is 30 times too low to account for the fact that the resorbate is isosmotic with the luminal contents.

If water is drunk, some hemodilution takes place, and this provides a signal for osmoreceptors in the hypothalamus and the liver (26, 27). This hemodilution results, in the first instance, not from the absorption of hypotonic fluid, but rather from a rapid initial passage of sodium chloride out of plasma into the lumen of the gut. This leads to a dilution of the portal blood leaving the splanchnic vessels. This ionic flux from blood to lumen diminishes in magnitude aborally so that it is least conspicuous in the large intestine.

Water is absorbed from the intestinal lumen mainly in association with the active transport of sodium, which has arrived there either from dietary intake or, as already mentioned, by efflux from the blood. The efficiency of this process is quite astonishing, when one considers the ratio of water to sodium transported.

The absorbate is isotonic, which means that 1 liter of water is transported for every 300 mMoles of salt. Since the molecular weight of water is 18, one liter represents about 55.6 moles. Hence, the transport of 1 mole of sodium chloride brings about the movement of $55.6/0.3 = 185$ moles of water.

For the absorption of water to occur in the distal ileum glucose must be present in the lumen. In contrast with most other epithelial cells the mucosal cells in this location have the property of taking up D-glucose passively from their luminal surfaces and channelling it into their metabolic pathways. It seems that in this part of the gut the energy for the sodium transport system is derived from the catabolism of the glucose which is taken up at the same time.

The composition of the intestinal absorbate, as well as the composition of the juices secreted, is different in different segments of the intestine. There is evidence (13) that the duodenal mucosa serves to establish equilibrium between chyme and blood by permitting rapid fluxes in both directions. The ileum, on the other hand, brings about a net absorption of sodium and water into the body. Throughout the length of the small intestine the active transport of sodium is accompanied by isosmotic transport of water.

In the ileum and jejunum, chloride is actively taken up in exchange for bicarbonate, so that the luminal contents become slightly alkaline. The colon similarly absorbs chloride by an active process and secretes bicarbonate and potassium. (This is the cause of hyperchloremic acidosis, which arises when the ureters are transplanted into the colon.)

The maximum rate of water absorption by the intestine lies well above what is normally required. The net absorptive rate is the difference between two opposing fluxes. It can be shown that 50 percent of a dose of isotopically labeled water can pass from the small intestine into the blood within 2 to 3 minutes, while about 30 minutes are required for the absorption of 50 percent of a given volume of water. The most recent findings (40) indicate that steady-state water reabsorption may reach 15 ml of water per minute, a value that—either by design or good fortune—is very close to the maximum attainable rate of urine flow.

In addition to ingested water the intestinal tract also reabsorbs considerable volumes of water derived from the secretions of the digestive glands. The total volume of fluids secreted into the gastro-intestinal tract each day is more than twice the volume of plasma from which it is derived. These fluids, with the nutrients and minerals dissolved in them, are completely reabsorbed by the normal gut.

The electrolyte content and considerable volume of the gastro-intestinal secretions are important in understanding the consequences of diarrhea, vomiting, and various surgical conditions. Because such large volumes of fluid are involved, grave disturbances of acid-base balance and significant shifts in electrolyte status can occur very rapidly when the gastro-intestinal contents are lost from the body. Accurate estimates of the amounts of fluid and electrolytes lost are therefore essential if appropriate replacement therapy is to be instituted.

The Body Fluids

The body fluids provide a typical example of a steady-state system so characteristic of living organisms. The several compartments of the body fluids are kept in a very constant condition as regards both their volume and the composition of their contents. This constancy is maintained despite a very extensive interchange between the different compartments and also with the external environment. The total water content of the body can be easily determined by the indicator-dilution method, which depends upon the relationship between the concentration of a test substance in a solution and the mass of substance present, *viz*:

$$C = \frac{M}{V} \quad \text{or} \quad M = VC$$

where V is the volume of solvent, C the concentration, and M is the mass of substance present.

Clearly, if two of these quantities are known, the third can be calculated. Thus, if one wishes to determine the total volume of the body water, one injects a known quantity (M) of tritiated water, and after allowing a certain time for it to mix with the water throughout the body, one takes a sample of plasma and measures the concentration of tritium (C). Since it is known that tritiated water does mix freely with all the water in the body (44), its "volume of distribution" (V) is equal to the total quantity of water in the body, i.e.,

$$V = \frac{M}{C}$$

It is important that the test or indicator substance possess the following properties:

1. Its distribution must be limited to the compartment being measured and must be uniform within that compartment.
2. The substance must not be injurious; neither can it be metabolized nor synthesized in the body.
3. If the substance is excreted, the amount excreted must be easily measured, so that a correction can be made. For such a substance the formula for calculating its volume of distribution is

$$V = \frac{M - E}{C}$$

where E is the quantity of substance excreted up to the time when the plasma sample was taken.

It is usual to administer the test substance either as a single injection or by means of continuous infusion. If a single injection is used, a number of plasma samples are taken, and the falling concentration of the substance is plotted against time on semi-logarithmic paper (see Fig. 52). After a rapid initial fall due to mixing (a to b), the points should fall on a straight line, the slope being determined by the rate of excretion, or catabolism, or both processes. When a sufficient number of points have been plotted to establish the slope of the graph, the straight portion can be extrapolated to zero time (the dotted line in Fig. 56a). This gives the concentration present if instantaneous mixing and no excretion had occurred.

When a continuous infusion is used, the test substance is introduced into the body until a stable concentration is reached in the plasma, a time that varies with the different test substances (Fig. 53). When the plasma

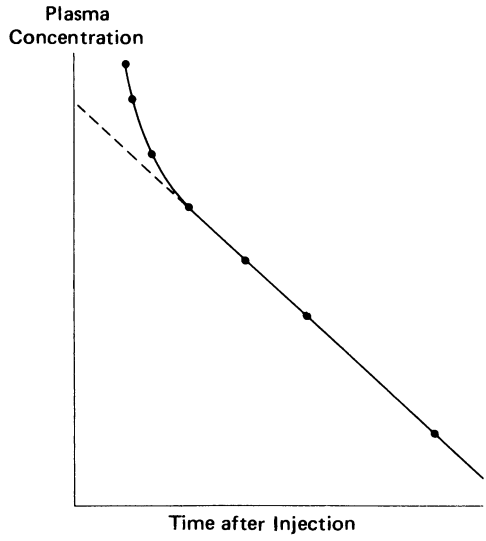

Fig. 52. Single-injection technique for estimating plasma volume or extracellular space.

concentration has settled at a steady value, the infusion is discontinued, and all of the test substance subsequently excreted is collected and measured. The total quantity of substance excreted then represents the quantity in the body at the time when the last blood sample was taken, and so is equivalent to M in the formula already discussed.

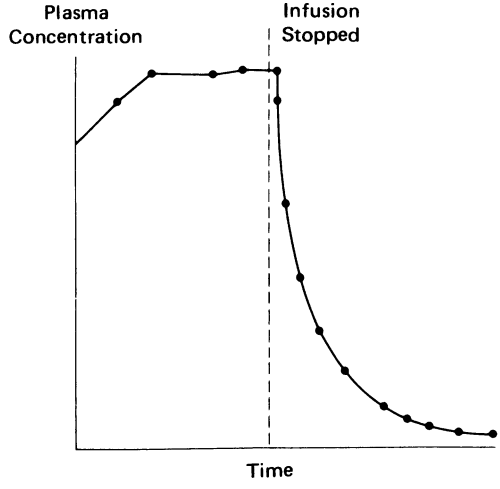

Fig. 53. Constant-infusion technique for estimating plasma volume or extracellular space.

The use of isotopic tracers as indicator-substances is presently common practice, and it is therefore pertinent to mention two special criteria to which such tracers must conform (29). These are:

1. The injection of the isotope must not influence the mechanisms normally governing the size and composition of the compartment to be measured.

2. The body must not be able to distinguish between the labeled and the unlabeled molecules.

An important assumption always made when measuring the body fluid spaces (i.e., compartments) is that the resistance to water movement between compartments is located at the interface between compartments; for example, at the cell membrane when one is concerned with intracellular fluid. Until recently nobody questioned that water entering the body mixed freely with all the water already present (17). Eighty years ago Pfeffer (48) postulated that the cell membrane was the only barrier to the movement of soluble substances and that the time required for intracellular diffusion was negligible by comparison. Since all cell membranes are regarded as freely permeable to water, the idea has grown up that water is rapidly and uniformly distributed throughout the body.

Certainly if one relies on these assumptions in calculating the fluid spaces of the body, no great error will be introduced. However, the assumptions are, in fact, not established beyond question. For example, Ling (38) believes that in the distribution of isotopic water, the rate-limiting factor is not permeation through the cell membrane but the speed of diffusion through intracellular water. The physical state of the water in a living cell may be responsible for some non-uniformity in the distribution of a tracer. On the basis of a demonstration that some of the water in erythrocytes is not accessible to solutes, Cook (14) concluded that a not insignificant fraction of intracellular water is present in a special form and is actually bound to protein. At a more elemental level Bentzel has presented evidence that a portion of mitochondrial water is not free to participate in osmotic processes (10). These considerations may lead to some revision of current ideas regarding the volume of the intracellular space, but for present purposes they may be neglected.

A summary of the measured values for total body-water expressed as a percentage of the body weight is shown in Table 8, which indicates the variations with age and sex. The high water content of children is due for the most part to a large extracellular space. The difference between men and women is largely attributable to differences in fat content. Since fatty tissue contains very little water, the accumulation of fat appears as a decrease in the percentage of water per body weight (34). If body water is expressed as a percentage of fat-free body weight, the differences between men and women disappear. For this reason data on body composition are

Table 8. Total body water as a percentage of body weight. From Moore (42) and Steele et al. (58).

Age	Males	Both sexes	Females
0–1 month		76	
1–12 months		65	
1–10 years		62	
10–16 years	59		57
17–39 years	61		50
40–59 years	55		47
60 years and over	52		46

often expressed in terms of the "lean body mass," so that the adipose tissue is excluded from the calculation.

The *volume of distribution of anti-pyrine* (the anti-pyrine "space") is usually somewhat smaller than the volume of distribution of titrated water, but both correspond reasonably well with earlier estimates of body water based on desiccation and calculation of the ratio of wet to dry weight.

The simplest subdivision of the body water is into an extracellular and an intracellular phase. Of these, the extracellular phase comprises the blood plasma and interstitial fluid, which constitute the immediate environment of the body cells (the "milieu intérieur") of Bernard (11). Within the cells in the intracellular fluid the principal metabolic reactions of the body take place. All traffic of nutriments, minerals, and hormones conveyed to the tissue in the blood stream must traverse the interstitial fluid in order to reach the cells. Metabolic waste products arising within the cells leave by the same route in the opposite direction. A small fraction of the interstitial fluid is continuously drained away through the lymphatic channels and is called lymph. Lymph has a higher protein content than the larger fraction of the interstitial fluid which returns to the vascular compartment through the walls of the blood capillaries (46).

Figure 54 shows the principal differences in chemical composition between fluids found in the major compartments of the body. Unlike the ameba, whose extracellular milieu is effectively of infinite volume, a mammal's interstitial fluid is relatively small as compared with its intracellular space, and therefore it needs to be continuously reprocessed if it is to maintain a stable composition. The most important organs concerned in this homeostatic control are the kidneys and lungs; the most important forces utilized are diffusion, active transport, and hydrostatic pressure, the last-named being provided by the beating of the heart.

Interstitial fluid is continuously delivered to the tissues by ultra-filtration from the plasma near the arterial ends of capillaries and is returned to

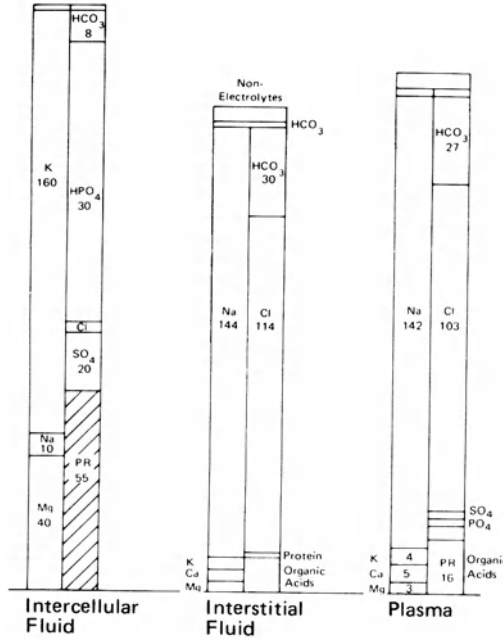

Fig. 54. Composition of the body fluids of the intracellular space, interstitial space, and plasma. The apparent differences in concentration are due to the varying quantities of protein present in each compartment. If the concentrations were expressed per unit volume of water, these differences would become very trivial. PR = protein.

circulation near their venous ends, where the hydrostatic pressure is less by some 10 to 15 mm than the oncotic pressure of the plasma. The forces responsible for these movements of fluid between the capillaries and interstitial space are the hydrostatic pressure built up by the contractions of the ventricles on the one hand and the oncotic pressure of the plasma proteins on the other. These oppositely directed forces were recognized by Ludwig in the case of ultra-filtration and by Starling in the case of reabsorption. The interaction of these forces is represented in a simplified way in Fig. 55.

Using values for capillary permeability derived from studies on other homoiotherms, the pressure differences shown in Fig. 54 could account for formation of some 20 liters of plasma ultra-filtrate per day in an adult man (45). The total lymph flow in a resting, fasting man is of the order of 2 liters per day, although it may rise, as a result of meals, to 3 to 4 liters per day. The remaining 16 liters of interstitial fluid must be returned to the capillaries. This 20 liters of interstitial fluid per day would not suffice for the metabolic needs of the cells, and so by far the greater proportion of nutrients must reach the cells by simple diffusion across the capillary walls,

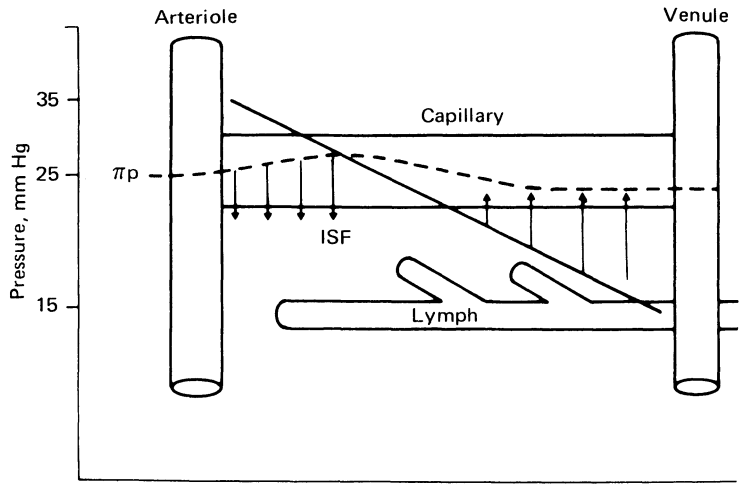

Fig. 55. The gradient of pressure from the arterial to the venous end of a capillary.

while metabolic waste products must be carried away by the same diffusional process. The necessary concentration gradients will be built up by the metabolic activity of the cells, resulting in the entrance of substrates on the one hand and the release of end products on the other.

The diffusion equilibrium between plasma and interstitial fluid provides the basis for the procedures used to determine the volume of the extracellular space. For estimating the volume of this compartment a substance is required that will pass through the capillary walls but will not enter cells. An ideal substance for this purpose has not yet been found; all the substances tested thus far enter the cells to some extent. The volumes of distribution of substances commonly used to determine the extracellular space give values of about 16 percent of the body weight in the case of inulin (35) and about 30 percent in the case of mannitol and the isotopes of Cl and Na. This difference results partly from the entry of the small ions into cells and partly from the fact that the relatively large inulin molecule diffuses only very slowly into the interstitial fluid of dense connective tissue. In the case of sodium there is also some incorporation of the isotope into the crystalline structure of bone.

The diversity of volumes of distribution of the various indicator substances used in the determination of the extracellular space are attributable to the fact that the extracellular space is itself a heterogeneous assemblage of different physiological entities. Among these one may distinguish (1) plasma; (2) interstitial fluid and lymph; (3) extracellular space of dense connective tissue, tendons, and bones; and (4) transcellular fluid.

The plasma volume and circulating blood volume can be determined by the dilution principle using an indicator substance that remains confined to the intravascular space. For many years the dye-stuff Evans Blue (which binds firmly and rapidly to plasma proteins) and radio-iodinated serum albumen (RISA) (47) have been used, since these large molecules move out of the capillaries at a relatively predictable rate. The volume of distribution can be estimated as before, by extrapolating the disappearance curve to zero time. Frequently an estimate will suffice, and in this event a single blood sample can be taken 10 minutes after the injection was given (to allow time for mixing). The concentration in the 10-minute sample corresponds tolerably well with that found by extrapolation. The blood volume can be calculated from the plasma volume by making use of the hematocrit. Since the contribution of the plasma to the blood volume is equal to (100 − hematocrit)/100, and since the total plasma volume is equal to the total blood volume times the plasma percentage,

$$\text{Total blood volume} = \text{plasma volume} \times \frac{100}{100 - \text{hematocrit}}$$

The blood volume can also be calculated if the total volume of circulating erythrocytes is determined—again by the use of the hematocrit. The circulating erythrocyte-volume is usually estimated by the carbon monoxide technique or by the dilution principle using ^{51}Cr or ^{32}P as marker.

It is interesting that the blood volume as determined by labeled red cells is always somewhat smaller than that determined by labeling the plasma proteins. This discrepancy is, however, so small that either method can be employed in practice without serious error (25).

The volume of the interstitial fluid and lymph must be calculated from the difference between total extracellular space and plasma volume. The measurement of the volume of the interstitial compartment is the most unreliable of all the space measurements, first because it includes any error in the plasma volume determination, and second because the indicator substances used distribute themselves unevenly through the various subdivisions of the extracellular space (see page 115). The best of these indicators are the sugars, which permeate rapidly into all regions of the extracellular space, exclusive of dense connective tissue and bone. Values obtained with such indicators correspond to about 12 percent of the body weightor 20 percent of total body water.

The bones contain very little water per unit weight, but because of the large mass of the skeleton, about 8 percent of the body water is associated with it. Dense connective tissue and cartilage have a very poor blood supply and contain very few cellular elements, so that the water of these tissues is largely extracellular. As has already been mentioned, it is penetrated only

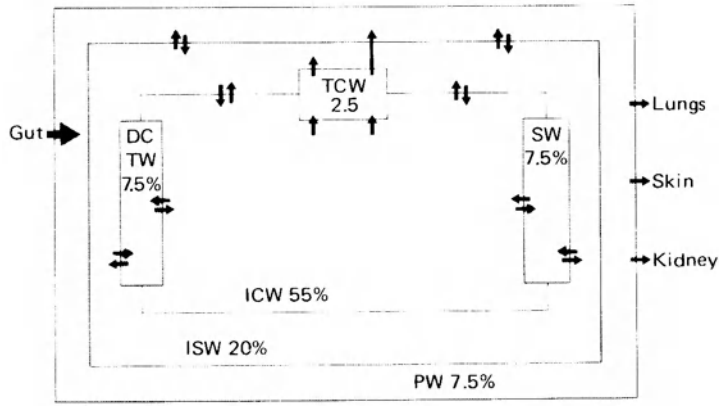

Fig. 56. The distribution of body water. ICW = intracellular water, ISW = interstitial water, P = plasma water, DCTW = water of the dense connective tissue, SKW = skeletal water, TCW = transcellular water.

very slowly by indicator substances, but these tissues together contain another 8 percent of the total body water (41).

The transcellular fluids are produced by active transport processes operating across epithelial membranes and they comprise glandular secretions, cerebrospinal fluid, and the ocular fluids. By far the largest volumes of transcellular fluids are secreted by the digestive glands, including the succus entericus. The composition of each of these transcellular fluids is specifically adapted to its function, and normally they account in all for about 2.5 percent of the total body water.

A simplified summary of the distribution of the body water is presented in Fig. 56 and Table 9, but it must be borne in mind that age, sex, and

Table 9. Distribution of the body water in a young man. After Edelman and Liebman (20).

Fluid compartment	ml/kg body weight	Percent of total body water
Plasma	45	7.5
Interstitial fluid and lymph	120	20.0
Dense connective tissue and tendon	45	7.5
Bone (slowly exchangeable)	45	7.5
Transcellular	15	2.5
Total extracellular	270	45.0
Total intracellular	330	55.0
Total body water (D_2O space)	600	100.0

adiposity introduce significant deviations from the mean values. The dilution techniques described as well as direct analysis of tissue samples have enabled us to define the fluid compartments of the body in health with considerable accuracy and to detect alterations resulting from disease.

Composition and Ionic Content of the Body Fluids

The ionic composition of the most important body fluids was set out in Fig. 54, (page 114). A Gibbs-Donan equilibrium (see page 3) exists between the diffusible ions in the plasma and in the interstitial fluid. The major difference in ionic composition between extracellular and intracellular fluids is maintained by active transport processes occurring at the cell membranes, whereby sodium is extruded from the cells and a high intracellular concentration of potassium is maintained. When, as a result of ion movements, osmotic gradients are set up, water passes through the cell membrane to equalize the total solute concentrations on the two sides. It is therefore obvious that the distribution of ions between the intracellular and extracellular phases is an important factor in controlling the absolute and relative sizes of the body-fluid compartments.

The total body sodium in an adult man amounts to about 60 meq/kg body weight, or 4000 meq in the whole man. This sodium is shared almost equally between the extracellular fluid (53 to 55 percent) and the bones (44 percent), the small residue of 2.5 percent being found intracellularly. The average sodium concentration in intracellular fluid is about 4.2 meq/l., but in certain tissues, such as renal cortex, it may be much higher. The fraction of total body sodium that equilibrates with isotopic tracers (^{22}Na or ^{24}Na) is called the exchangeable sodium (7, 19, 42, 50), and includes the extracellular sodium, intracellular sodium, and about one-quarter of the sodium of bone. The remaining three-quarters of the bone sodium is firmly bound within the crystalline structure and is not exchangeable (16). All exchangeable sodium is in diffusional equilibrium with the plasma sodium and so is available to be drawn upon as a reservoir when excessive sodium losses occur; for example, as a result of heavy sweating, diarrhea, or acidosis. The distribution of the body sodium in a healthy young man is indicated in Table 10.

The body potassium has been estimated by both direct analysis of the separate organs and whole-body counting of the naturally occurring radioisotope ^{40}K. It amounts to 42 meq/kg body weight, and the exchangeable potassium measured with ^{42}K is of the same order, which means that, in contrast to sodium, virtually all potassium in the body is freely exchangeable (22, 39, 50). There are, however, fractions that exchange only rather slowly, and these are found in the erythrocytes, brain, and bone. Neverthe-

Table 10. Distribution of the body sodium in a young man. After Edelman and Liebman (20).

Fluid compartment	Meq/kg body weight	Contribution to body's exchangeable Na, %	Contribution to body's total Na, %
Plasma	6.5	15.9	11.2
Interstitial fluid and lymph	16.8	41.0	29.0
Dense connective tissue and tendon	6.8	16.5	11.7
Skeleton (total)	25.0	19.5	43.1
Skeleton (exchangeable)	[8.0]	[19.5]	[13.8]
Transcellular fluid	1.5	3.7	2.6
Total extracellular Na	56.6	96.6	97.6
Total intracellular Na	1.4	3.4	2.4
Total body Na	58.0	141.0[b]	100.0
Exchangeable extracellular Na	39.6[a]	96.6	68.3
Exchangeable intracellular Na	1.4	3.4	2.4
Total exchangeable Na	41.0	100.0	70.7

[a] 56·6 − (25 − 8).

[b] $\frac{58}{41} \times 100$.

less, after a lapse of 48 hours, 90 percent of total body potassium has equilibrated with the injected isotope (44). The distribution of potassium in a healthy young man is indicated in Table 11.

Variations in body potassium occur according to age and sex and are well correlated with the fat content of the body, so that the exchangeable potassium provides a good index of the "fat-free cell mass."

Only 2 percent of body potassium is present in the extracellular space at a concentration of 4 to 5 meq/l., and in fact the extracellular (or plasma) concentration is a very poor indicator of intracellular potassium stores. Only when the plasma concentration changes by 60 percent or more can one be sure that this reflects the overall potassium status of the individual, and by then cardiovascular and neuromuscular disturbances are often present.

Calcium is the most important constituent of bones and teeth, and because of the great weight of the skeleton, the calcium content of the body is about 800 meq/kg body weight (62). Apart from the bones, intracellular calcium is found in other tissues in only traces. In plasma the concentration is about 5 meq/l., while interstitial fluid contains only about 2·5 meq/l., the

Table 11. Distribution of body-potassium in a young man. After Edelman and Liebman (20).

Fluid compartment	Meq/kg body weight	Contribution to body's exchangeable K, %	Contribution to body's total K, %
Plasma	0.2	0.41	0.37
Interstitial fluid and lymph	0.5	1.03	0.93
Dense connective tissue	0.2	0.41	0.37
Transcellular fluid	0.5	1.03	0.93
Skeleton	4.1	—	7.6
Total extracellular K	5.5	2.9	10.4
Total intracellular K	48.3	—	89.7
Total body K	53.8	110.0	100.0
Exchangeable extracellular K	1.4	2.9	2.7
Exchangeable intracellular K	47.0	97.1	87.3
Total exchangeable K	48.4	100.0	90.0

difference being due to the high protein content of plasma, where about 30 to 35 percent of calcium is protein-bound. Another 5 to 10 percent is bound to organic acids, and only 50 to 60 percent is in free ionic form. Calcium lost from extracellular fluid can be made good by drawing on skeletal stores.

Since in recent times more attention has been given to the physiological role of magnesium, its distribution has been carefully examined. The total content in the body is about 15 meq/kg. As with calcium, only the ionized fraction is physiologically important in the body fluids. Experimental poisoning with excess magnesium leads to central nervous depression and also some depressant effect on the heart. A fall in the plasma magnesium leads to neuromuscular hyper-excitability, which may go on to tetany.

Chloride is the most prominent anion in the extracellular fluid (61). Red blood cells contain chloride, as do the cells of the gastric mucosa which secrete hydrochloric acid. On the other hand, connective tissue and skeletal muscle contain very little chloride, but intracellular chloride is, in general, several times higher than intracellular sodium. Total body chloride has been determined in several warm-blooded species, using the isotope dilution principle, and an average value of 33 meq/kg body weight has been reported. As in the case of sodium, the figures are higher in men than in women. Movement of chloride into and out of the body is closely coupled with that of sodium and is influenced by the same factors as those effecting sodium balance. In many disturbances of acid-base balance, changes in chloride concentration show a reciprocal relationship to changes in bicarbonate.

Exchangeable bicarbonate amounts to about 10 to 12 meq/kg body weight and is almost equally partitioned between intracellular and extracellular fluid. The total quantity of body bicarbonate is much greater than the exchangeable quantity because of the large deposits of carbonate in the bones, which can be released only by strong acids. Bicarbonate in the plasma and interstitial fluids has, of course, a very important buffering function. The pK of a bicarbonate solution is the basis of the characteristic pH of mammalian blood, and the extracellular store of bicarbonate constitutes the first line of defense in the event of hydrogen ion excess within the body.

Volume Regulation in the Fluid Compartments and Sodium Balance

The size of a living organism is a characteristic of the species and the individual. In an adult man the body weight remains remarkably constant, regardless of great variations in sodium and water intake. Since living organisms consist in large part of water, it follows that the volume of body water is controlled within very narrow limits, and in fact this is one of the most important homeostatic mechanisms known.

Not only is the volume of total body water kept as constant as possible, but so is the total body water of its separate compartments. This is especially evident in the case of the blood volume. As described on page 115, plasma forms a functional continuum with interstitial fluid, and the distribution of water between these compartments is dependent solely upon hydrostatic and oncotic pressure gradients operating across the boundary between them, i.e., the capillary wall. If the balance is upset by changes on one side of this boundary, it is modified by transfer of fluid across the boundary. Hence, if water is absorbed from the gut and enters the plasma, some of it will pass into the interstitial space until equilibrium is restored. Schwiegk (55) has introduced the term "overflow tank" to describe this function of the interstitial space. But the interstitial space also acts as a reservoir under conditions of water deficit when, either as a result of copious sweating or some pathological process, plasma volume is reduced. In these circumstances the hydrostatic pressure within the capillaries falls and the osmotic pressure of the plasma proteins causes water to be sucked into these vessels from the interstitial space.

Along with water movement, there must necessarily occur a further important physical consequence, *viz.*, a change in the osmolarity of the body fluids. When pure water is drunk, the concentration of solutes in the extracellular space is reduced, but since cell membranes are more permeable to water than to any other substance, the cells will also take up water. Thus,

if the vascular system is overloaded with water, there is a danger of a general reduction in osmolarity throughout all the body fluid compartments. If there were not an efficient mechanism acting to bring about the excretion of excess water, the overloading of the vascular system would, of itself, lead to venous engorgement and possibly to water intoxication occasioned by the general dilution of body fluids.

Both of these dangers—hypervolemia and hypotonicity—are normally averted by a physiological adjustment, *viz.*, the onset of water diuresis (see page 87). There exist in warm-blooded animals sensory receptors that respond to both types of disturbance by inducing water diuresis. Receptors signaling hypervolemia are located in the low pressure areas of the circulatory system, at the entry of the pulmonary veins into the left atrium, and the osmoreceptors that signal hypotonicity are located in the hypothalamus. Excitation of either set of receptors leads to an inhibition of the production of ADH in the supra-optic and para-ventricular nuclei (see page 88). This inhibition of the output of ADH results in a water diuresis, i.e., the excretion of osmotically free water. The two mechanisms can be seen operating in the experiment recorded in Fig. 57, which was carried out on an unanesthetized

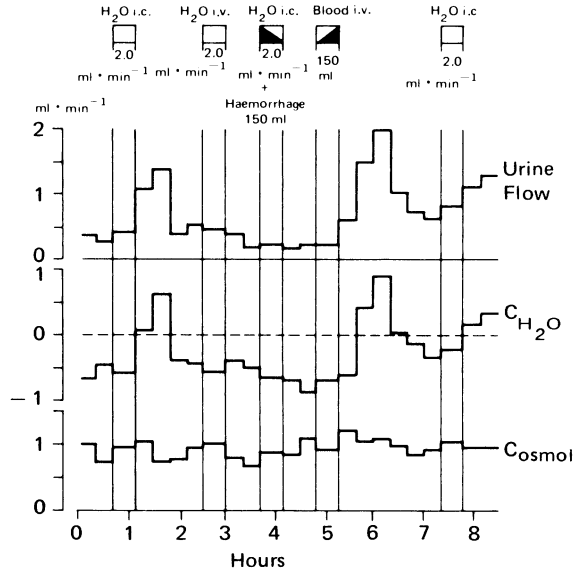

Fig. 57. Effects on urine flow and free water clearance (CH_2O) of three intracarotid infusions of distilled water (H_2O i.c.) at the rate of 2 ml/min for 30 min. The infusion of water accompanied by the withdrawal of 150 ml of blood produced no diuresis (◹). Restoration of the blood volume was followed by an increased urine flow (◪). Intravenous infusion of water (H_2O i.v.) was without effect. From Andt (3).

dog. When the osmotic pressure of the carotid blood is reduced by an infusion of water a water diuresis ensues. An intravenous infusion of the same volume of water is without effect, because the water is then distributed throughout the blood stream so that no adequate stimulus reaches the osmoreceptors. If the intracarotid infusion of water is accompanied by an acute experimental reduction in blood volume, water diuresis is prevented (fourth hour). This shows that the hypovolemic stimulus is more powerful than plasma hypotonicity in controlling the output of ADH. If blood volume is restored by transfusion, water diuresis follows (fifth hour).

When water is taken by mouth, the water diuresis that occurs can similarly be explained on the basis of a reduction in plasma osmolarity, just as in the case of an intracarotid infusion, as can be seen from Fig. 58. However, an oral load of isotonic salt solution is not excreted nearly so rapidly, because the relatively small increase in blood volume produced is insufficient to excite the atrial receptors, and of course, there is no change in tonicity of the plasma. If isotonic salt solution is infused intravenously, it is excreted almost as rapidly as an equal volume of pure water, and in all events if the experimental animal is sodium-deficient, a true water diuresis with hypotonic urine is produced (see Fig. 59). In this case a pure hypervolemic response is seen, but if the animal is not sodium-deficient, a simultaneous excretion of sodium obscures the picture.

It may seem curious that terrestrial creatures have evolved only a water-retaining hormone and not a water-eliminating hormone, but a consideration of their evolutionary history provides some enlightenment. If one accepts that our ancestors invaded the land from fresh water (56),

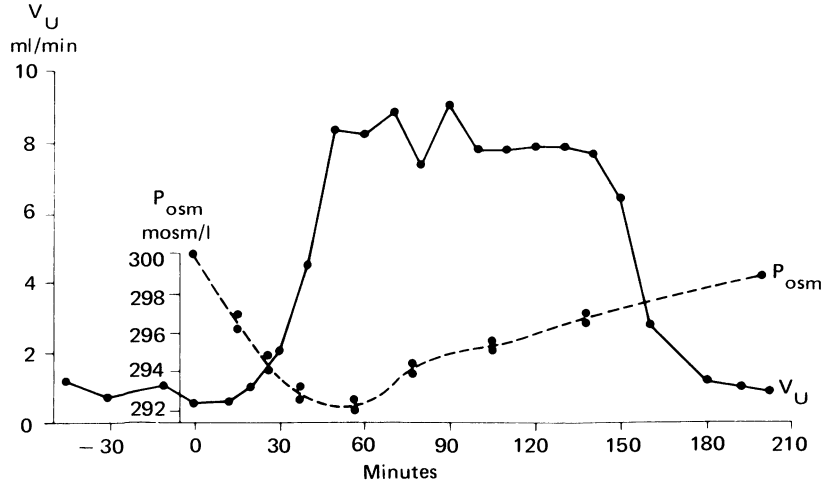

Fig. 58. Water diuresis in man following the ingestion of 1 liter of water at time zero. The rise in urine flow follows a fall in plasma osmolality.

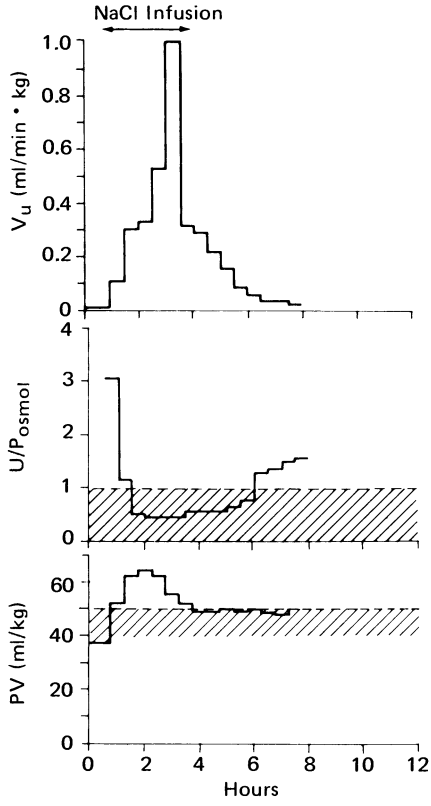

Fig. 59. Effect of infusion of isotonic NaCl solution into a sodium-deficient dog. The infused water is excreted, leading to hypotonicity of the urine. During infusion the previously contracted plasma volume (PV) rises above the normal value. It appears that ADH-release is inhibited for four hours by activation of a volume-sensitive pathway. From Keck et al. (32).

then clearly they came from an environment in which there was no shortage of water. On the contrary they would have been taking in water continuously (just like present-day freshwater fish and amphibia, through gills and skin) because of the osmotic gradient between their surroundings and their body fluids. This surplus of water is excreted by the kidneys in the form of very dilute urine, which is elaborated in their relatively long distal tubules. The ability to excrete dilute urine was developed quite early in the evolutionary story, and depended solely on the characteristics of the distal nephron, without any hormone being required.

The first land vertebrates, amphibia, were unable to make good the considerable losses of water that took place through their skin by drinking, since they had not yet evolved a thirst mechanism. They were obliged

therefore to return to the fresh water of the lakes and rivers from which they had emerged in order to replace their water losses by imbibition through the skin. In order not to be completely at the mercy of water losses through their skins, which limited the duration of their excursions on land and at the same time restricted these adventures to the immediate vicinity of water sources, they developed a water-conserving mechanism, located in the urinary bladder, which could be filled up while they were in water and emptied with the aid of the antidiuretic hormone into the blood stream while on land. On this basis it is understandable that the vertebrates, as they progressed through further evolutionary stages, had no need of a diuretic hormone. By operating an inhibitory control over the water-conserving hormone (i.e., ADH), they were able to put themselves back in the situation of their original aquatic forebears.

The position is quite similar with regard to sodium balance. Here, however, the development of a physiological control mechanism begins with the fishes rather than in terrestrial vertebrates. Freshwater fishes were faced with the necessity of excreting water that gained access to their bodies through the gills, and this excretion had to take place without loss of sodium, since there was comparatively little sodium to be obtained from their food. Hence, survival favored species in which a sodium-retaining hormone was evolved, *viz.*, aldosterone. In more primitive forms this hormone is secreted by the inter-renal body, but in more advanced creatures this function is carried out by the suprarenal cortices. Aldosterone increases the tubular reabsorption of sodium. In land animals, with their frequent opportunities to take in dietary sodium, it became advantageous to regulate the output of aldosterone so as to permit the excretion on occasion of large quantities of sodium.

The maintenance of the volume of the body fluids in man at an optimal level is dependent on an adequate intake of salt. If salt intake is restricted, the body fluids shrink proportionately and may undergo a reduction in volume of 3 to 4 litres. If salt is totally absent from the diet, renal and extrarenal losses continue at the rate of 0.5 meq Na/kg body weight per day, and in these circumstances some contraction of the fluid compartments is inevitable. In order to prevent such contraction in the dog it is necessary that the daily intake of sodium exceed 0.5 meq kg/day (49). Man, on the other hand, is able to stay in sodium balance on a diet virtually free of salt. Some initial weight loss is experienced before a new steady-state of ECF volume is reached, but then the ideal ECF volume in man has not been defined.

As has already been mentioned, aldosterone increases the renal reabsorption of sodium in adrenalectomized animals, but, in fact, exogenously administered aldosterone also reduces sodium excretion in animals with intact suprarenal glands, so that in the presence of endo-

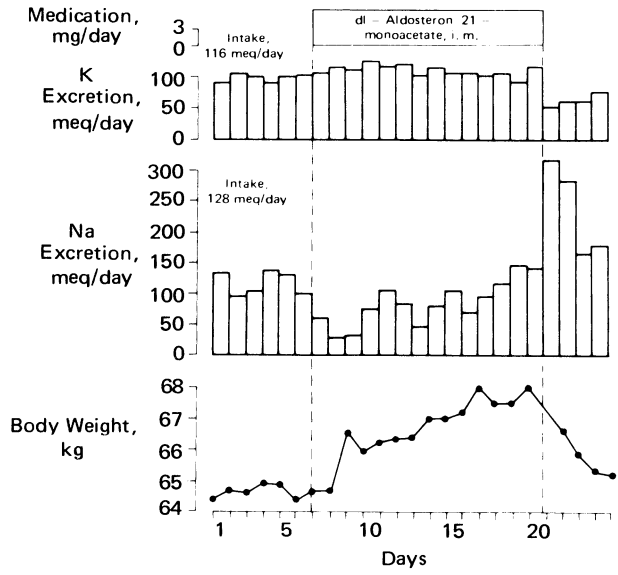

Fig. 60. The effect of aldosterone administered parenterally (3 mg/day) on the body weight and the excretion of Na and K in a healthy human subject on a constant intake of these electrolytes. It will be noted that during the first week on aldosterone Na and water are retained, but this effect levels off during the second week of treatment, with body weight becoming almost constant and Na excretion approaching daily intake. The excretion of K shows a slight rise during treatment (see page 37). After withdrawal of aldosterone the excess of retained Na and H_2O is excreted, and K excretion falls to restore the deficit incurred during administration of the hormone. From August and Nelson (8).

genous aldosterone the kidney will still respond for a time to additional hormone. With prolonged administration of aldosterone, however, sodium excretion returns to normal (Fig. 60). The volume of extracellular fluid then becomes stabilized at a new raised level. The term 'escape phenomenon" has been used to designate this state of affairs by which it is implied simply that the sodium excretory mechanism in the kidneys appears to have "escaped" from the influence of administered aldosterone. It may be supposed that when such "escape" occurs, the volume of the extracellular body fluids has reached an elevated but limiting value. This appeared to be the case in experiments on dogs in which the ECF volume had been increased by giving large amounts of salt and in which the "escape" phenomenon developed sooner the higher the salt intake (Fig. 61).

After withdrawal of aldosterone (and often while it is still being given), the excess of sodium retained earlier during the period of treatment is

excreted along with the simultaneously retained water, but the mechanism underlying these events is unknown.

It is noteworthy that in patients in whom, as a therapeutic measure, both adrenal glands have been removed, sodium balance can be maintained by a combination of the glucocorticoid hydrocortisone and a salt supplement of about 130 mMoles/day. If, then, a mineralocorticoid with aldosterone-like activity (deoxycorticosterone acetate or DOCA) is administered as well, the kidneys will excrete less sodium and body weight will increase, indicating a retention of water along with sodium. Here again an "escape" is observed insofar as the sodium excretion reverts to the control value although body weight does not. Apparently such a patient must have had a reduced ECF volume before the minerolocorti-

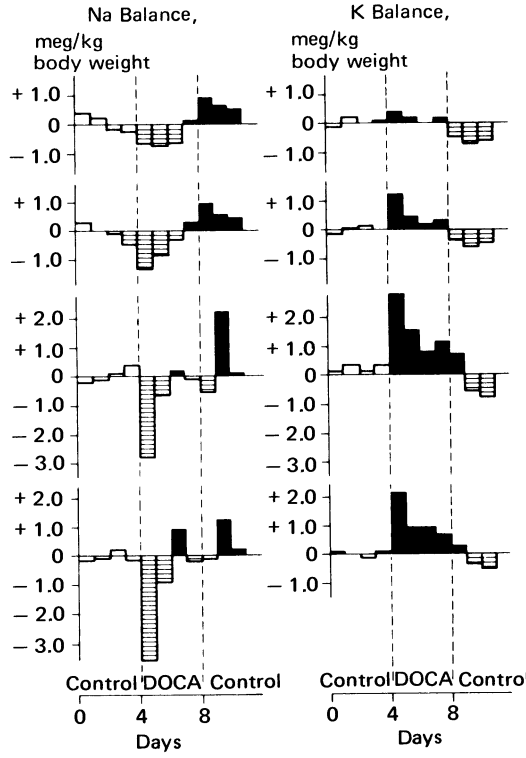

Fig. 61. Effects of an aldosterone-like mineralocorticoid (DOCA) on the excretion of Na and K in the presence of varying intakes of sodium chloride. Four groups of dogs (represented from above downward) received 2.5 meq/kg, 5.5 meq/kg, 9.0 meq/kg, and 15.3 meq/kg of sodium, respectively, with their daily diet along with 1.7 meq K/kg. During a period of four days the dogs received 25 mg/day of deoxycorticosterone acetate (DOCA). The higher the sodium intake, the shorter was the period of retention (-meq Na) and the more rapid was the onset of "escape" with net excretion of sodium. From Ellingham (21).

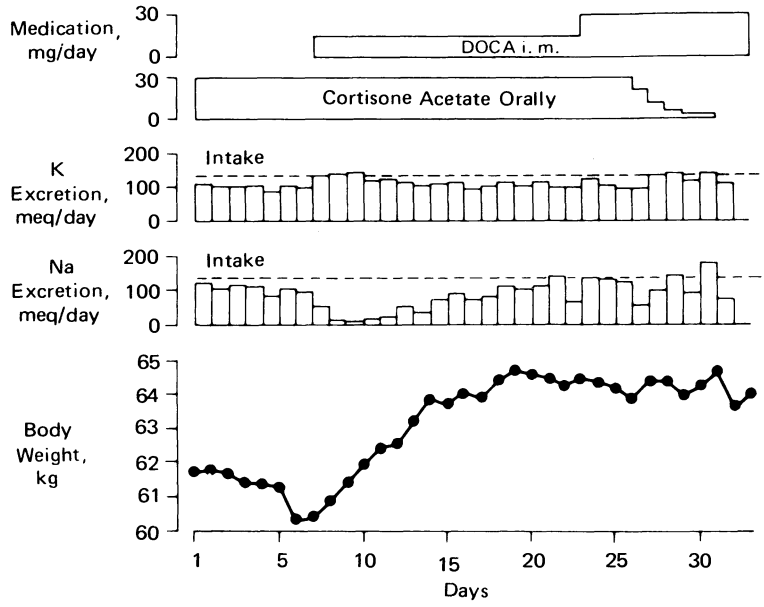

Fig. 62. Effects of cortisone and DOCA (deoxycorticosterone) on a bilaterally adrenalectomized human patient. While receiving cortisone and 120 meq Na/day in his diet, the patient remains nearly in balance with respect to Na and K, but body weight declines. When DOCA is given sodium retention occurs for about a week and body weight rises perceptibly. After 11 days on DOCA a pseudo-equilibrium is established which is unaffected by a doubling of the dose of DOCA, even when this is combined with a reduction in the dose of cortisone. From August and Nelson (8).

coid was given, and this is restored to its optimal level under the influence of DOCA (Fig. 62).

The findings reproduced in Figs. 60 and 62 demonstrate that the adrenal cortices have an important regulating effect on the volume of body water.

In view of experiments on dogs in which it was shown that massive infusions of sodium chloride lead to considerable increases in sodium excretion, despite simultaneous treatment with aldosterone, the existence of a "natriuretic hormone" has been postulated. This hormone is supposed to be elaborated through some mechanism, as yet unknown, in the presence of an expanded ECF volume and to have the ability to inhibit sodium reabsorption in the nephrons. Conclusive proof of the existence of this hormone is lacking at the present time, and the observed events that follow high salt intake plus aldosterone can be explained on the basis of the changed conditions (created by volume expansion) under which sodium reabsorption is taking place.

Survival in Conditions of Water Shortage

Man is less tolerant of water deprivation than most land animals. The maximum weight loss from desiccation compatible with life is in man about 20 percent of his normal body weight, compared to 30 percent for rats and mice, 40 percent for dogs and cats, 50 percent for birds and reptiles, and 60 percent for earthworms. Actually the rapidity with which dehydration develops is as important as its extent. For example, when a frog is dehydrated slowly, it will survive a 40 percent reduction in body weight, but if dehydration takes place rapidly, only a 12 percent reduction is tolerated (2, 28, 53, 54). The limit for survival time of man without water is, under the most favorable conditions, (i.e., in shady surroundings and at a moderate environmental temperature), between 11 and 20 days. Walking in a desert under direct sunlight and in a hot climate, survival is limited to a matter of hours, because the rate of water loss under such conditions may reach a liter per hour (2). Symptoms of water deficiency come on rapidly in tropical conditions but rather more slowly in temperate regions. Adolph (2) in *The Physiology of Man in the Desert* reports that weakness rather than thirst is the most common early symptom of men walking in the desert with insufficient water. At a water deficit of about 3 percent, salivary secretion and urine volume diminish, but even when the fall in body weight exceeds 5 percent, no serious disturbances occur although the heart may accelerate somewhat, the feeling of lassitude may increase, and rectal temperature may rise by about 0.5 or 1°C (37). When the loss of body weight exceeds 10 percent, some failure of mental powers as well as of physical strength may be noted; by the time weight loss has reached 12 percent, swallowing becomes impossible. Depending on the rapidity with which dehydration has occurred and on the climatic conditions, death occurs when weight loss is somewhere between 15 and 20 percent (2, 36, 60, 63). The cause of death from dehydration is not entirely clear, and although circulatory failure due to reduction in blood volume is obviously an important contributor, Kerpel-Fronius (33) ascribes the principal blame to cellular damage resulting from hypertonicity of the tissue fluids.

Physiological Effects of Dehydration

The physiological effects of controlled dehydration accompanied by graded muscular exercise have been extensively studied. Acute dehydration to the point of a 5 percent weight loss increases body temperature by 0.5°C and the pulse rate by about 30 beats per minute when light muscular exercise is undertaken (Fig. 63), the tachycardia being presumably due to a reduced intrathoracic blood volume. At the same time the maximum

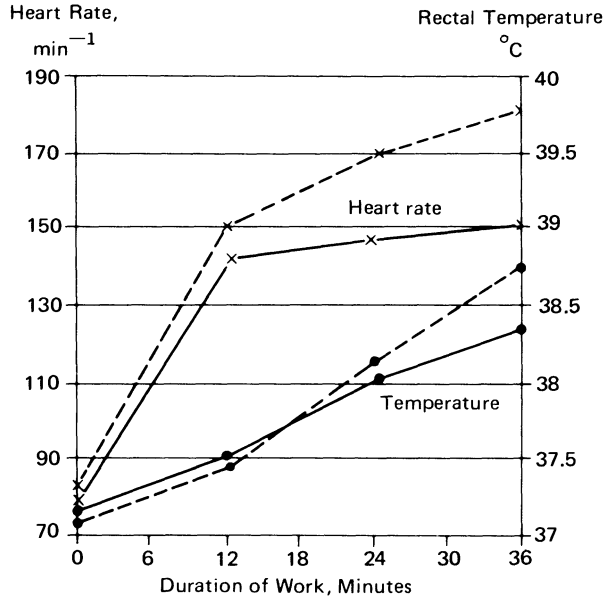

Fig. 63. Heart rate and rectal temperature in a man undertaking graded exercise. × : pulse rate. ●: rectal temperature. —: while in water balance. – – – : with a water deficit equivalent to 5 percent of the body weight. After Ladell (37).

oxygen uptake is reduced by about 200 ml/min. As may be seen from Fig. 62, neither the curve representing the body temperature nor that describing the heart rate has any tendency to plateau during the course of exercise in a dehydrated subject. This inability of a moderately dehydrated person to attain a steady state while performing light work accords with the general experience that under unfavorable environmental conditions, even a relatively slight water deficit carries with it some risk because within a few hours physical exertion may become impossible. A further illustration of the dangers of dehydration is provided by the susceptibility of infants to this condition. An infant weighing 5 kg contains about 3.5 liters of water. Its daily fluid intake of 800 ml corresponds to about one-quarter of its total body water and about one-half of its extracellular fluid. The turnover of body water in an adult on the other hand (about 2 liters) corresponds to only 5 percent of total body water, or 12 percent of extracellular fluid. Hence, any excessive water loss in the infant, whether due to vomiting, diarrhea, or sweating, leads to a serious degree of dehydration about five times more quickly than in an adult.

Survival at Sea

A man in a small boat or raft on the open sea is in as difficult a situation as one stranded in the desert. The only advantage the sea-borne castaway has is that in a hot climate he can moisten his clothing with sea water and so cool himself without the necessity of sweating profusely. Contrary to popular belief, very little water is absorbed via the skin.

The drinking of sea water is not to be recommended for the following reason. The salt content of the great ocean waters may be as much as $3\frac{1}{2}$ percent, and the human kidney cannot elaborate a urine with a salt content greater than about 2 percent. One may therefore calculate that for every 100 ml of ocean water drunk, at least 175 ml of urine must be passed in order to prevent salt retention, from which it is clear that a net loss of 75 ml of water would be incurred. In addition, the magnesium content of sea water provokes diarrhea with further loss of water in the feces.

Although experience attests to the fact that castaways who do drink sea water die more quickly than those who do not, papers appear in the literature from time to time which claim that sea water has life-saving value (12). There is, in fact, some foundation for these claims. One such is that certain seas, such as the Black Sea and the Caspian Sea, have only half the salinity of the Atlantic Ocean; the Baltic Sea is almost isotonic. Furthermore, in hot climatic conditions, as the crew of the Kon-Tiki found (30), salt losses due to sweating were so marked that small quantities of sea water were beneficial when consumed along with the freshwater ration.

The eating of raw fish is without benefit to the water economy of the body, because the whole of the water contained in fish is needed to excrete the salt and protein catabolites derived from its flesh.

Desalination of Sea Water for Drinking Purposes

Various types of distillation apparatus, of which some utilize the heat of the sun, have been developed in order to provide fresh water for the occupants of lifeboats and life rafts. These devices, owing to their bulk, complexity, and low yield, have largely given place to chemical methods of purification. The development of chemical procedures has been greatly simplified by two observations. First, water containing 0.3 to 0.4 percent NaCl not only tastes better than distilled water, but also is more suited to the body's needs in that it results in greater water conservation. Secondly, the sulphate present in sea water (about 50 meq/l.) is harmless to man. In view of these facts, the so-called Permutit method of desalination has been introduced.

The basic equipment consists of a tablet of silver zeolite about the size of a cigarette pack and a plastic bag with a built-in filter. Silver zeolite is a form of hydrated aluminium silicate ($Ag_2Al_2\ Si_3\ O_{10}(H_2O)_2$). The tablet is broken up and placed in the plastic vessel, which is filled with sea water: silver chloride and sodium and magnesium zeolites are precipitated out, and water suitable for drinking is sucked through the filter.

Water Supplies in Outer Space

Manned flights to the moon, and possibly in the future to the planets, pose new problems with regard to water supplies and water conservation. Because of the limited payload, the transport of large quantities of water is out of the question, so that the only practicable expedient is to recover the water excreted by lungs, skin, and kidneys. Among the most promising methods for reclaiming urinary water involves freeze-drying followed by filtration through activated charcoal. Another possible method is vacuum-distillation followed by passage of the vapor over a heated catalyst to combust organic contaminants. The absolute dependence of man on an adequate water supply may ultimately prove to be the decisive limiting factor to the exploration of outer space.

A further problem in ensuring water balance in space is of a regulatory nature. As a result of weightlessness, the distribution of blood in the body is similar to that in a supine subject on the earth's surface. The displacement of blood from the lower extremities, which normally occurs when the posture is changed from the upright to the supine, is continuously present in the weightless state, so that the volume receptors must be stimulated continuously (23, 24). Consequently the formation of ADH will be continuously inhibited, and water diuresis will occur. Simultaneous excretion of sodium will lead to a reduction in blood volume, and will, if uncorrected, limit the ability of the circulatory system to respond to any special demands imposed upon it.

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