Excretion

Introduction

A number of waste products are formed in animals during the metabolic activities of the body in addition to the release of the energy required for various life activities. These waste products become toxic or harmful if retained for prolonged time periods or exceed some concentration limits. A byproduct of metabolism under certain conditions may be essential for the body and the same substance under other conditions may become a waste product to be eliminated by the body. Water is a typical example. It is a byproduct of metabolism which is usually retained by the body but under condtitions of edema, it is a waste product and is eliminated. Glucose is another example. Carbon dioxide is a metabolic end product which is usually excreted but it is an important component to maintain acid-base balance in the body. Urea is a byproduct in nitrogen metabolism. If the blood urea level exceeds 0.05%, uremia develops leading to severe toxic conditions. However, urea is actively conserved in elasmobranch fishes to maintain osmoregulation. Thus, a particular substance may be a waste product in one animal but may be an essential substance in other animals. The end products as well as byproducts of metabolism are eliminated from the body in aqueous solutions, therefore water constitutes the bulk of the excreta.

Excretion plays an important role in maintaining the homeostatic condition of the body. Failure of excretory system results in accumulation of waste products, leading to disturbances in osmoregulation, ionic regulation, acid-base balance and ultimately to death.

In single celled animals, no special excretory system is found as the waste products formed in the body together with water are released through the general body surface into the surrounding medium. But in multicellular animals the situation becomes complicated because all cells are not in direct contact with the outside medium and they thus can not release the products through the body surface. This has necessiated the development of the excretory system which separates the waste products form the circulating medium of the body and releases them outside the body. In general, the tissue fluids accumulate the waste products from cells and pass them on to the circulating medium like blood. Blood in turn transports these excretory products to the excretory organs which discharge them outside the body.

All the waste products removed from the body are not in general inculded in the process of excretion. It is the usual practice to include **removal of nitrogenous waste products from the body** which constitutes the process of excretion. The term excretion means **separation and elimination of the unwanted waste materials from the body.** The waste materials are of several kinds and vary from animal to animal and also in the same animal from time to time.

Byproducts of Excretion

During the process of metabolism many byproducts are produced. Some of these compounds are useful to the body and others are to be removed.

Water. Most of the animals, whether they live on land or in water, take large quantity of water with their food. The birds and mammals drink it as well. Some water is used in the complete oxidation of fats, carbohydrates and in all classes of reactions called condensation. Further, the surface of the animal cell is more or less permeable to water. If the osmotic pressure of the protoplasm is greater than that of fresh water as found in fresh water animals, water will enter in the animal body osmotically. Moreover, the cell membrane of the animal cells are not rigid like those of plants. So this process of osmosis will go indefinitely. In such animlas, the regulation of water is more important than excretion.

Carbon dioxide. Carbon dioxide is normally the end product of those substances which contain the carbon in the process of oxidation. The organ system, which excretes it, is generally that which is ventilating the body with oxygen; so its excretion is considered under respiration. Only small quantities of CO_2 is consumed (removed) by other ways i.e. converting into urea, $CaCO_3$ or $NaHCO_3$.

Nitrogen compounds. Nitrogen compounds are more important than the other groups and are removed in manys ways. Fat and carbohydrates are oxidized to form water and CO_2 which are readily excreted in many ways and nucleic acids contain nitrogen, which is excreted in many ways and as such various compounds are formed according to the physiology of organism. Nitrogenous wastes may come from proteins (by way of amino acids) or from the purines and pyrimidine bases of nucleic acids and from other miscellaneous nitrogenous compounds.

Chemical Nature of Nitrogenous Products

Different types of nitrogenous products are formed in the animals depending upon their nitrogen metabolism. The following are the main nitrogenous excretory products met in different animals :

1. Amino acids. As a result of digestion, proteins are hydrolyzed into their respective amino acids. Excess of amino acids is excreted as such without any change by certain animals such as Molluscs *(Unio, Limnaea)*, Echinoderms *(Asterias , Paracentrotus)* etc.

2. Ammonia. Ammonia is formed as a result of the deamination of amino acids as follows :

$$R-\underset{\displaystyle COOH}{\overset{\displaystyle NH_2}{\underset{|}{\overset{|}{C}}}}-H+\tfrac{1}{2}O_2 \longrightarrow R-\overset{\displaystyle O}{\overset{\|}{C}}-COOH+NH_3$$

$$R-\underset{\displaystyle COOH}{\overset{\displaystyle NH_2}{\underset{|}{\overset{|}{C}}}}-H+H_2O \longrightarrow R-\underset{\displaystyle COOH}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}-H+NH_3$$

Ammonia is a poisonous (toxic) substance and it is present only in low concentration in the blood of many animals. It is formed rapidly in the drawn blood of vertebrates but the amount present in mammals is probably not more than 0.001 to 0.003 mg/100 ml of blood. The concentration of NH_3 in blood of amphibians, reptiles and fishes is higher than that of the mammals. But it is less than 0.1 mg/100 ml. Because of its toxic nature NH_3 must be eliminated out from the blood rapidly or it is to be changed into lesser toxic substances. Ammonia is highly soluble in water and is excreted as such by many animals, which have ample water for carrying away the excretory porducts.

3. Urea. The excretory product is less poisonous or toxic and is more soluble in water than NH_3 .The liver, in many animals, contains an enzyme *arginase*, which is responsible for the formation of urea by the process of catalysis from the amino acid arginine. There are evidences to show that the conversion of ammonia into urea is by a cyclical chain of chemical reactions in the liver, known as *Kerb's cycle* or *Ornithine cycle.*

4. Uric acid. Uric acid is present as a nitrogenous product among the birds, terrestrial reptiles, some snails and insects. In this case, ammonia is converted into uric acid. Uric acid is less toxic and insoluble in water. It can be stored and excreted in crystalline form and hence requires minimal amount of water for its elimination. The exact mode of formation of uric acid from NH_3 is not known.

5. Trimethylamine oxide. This nitrogenous product occurs in large quantities in marine teleost fishes.

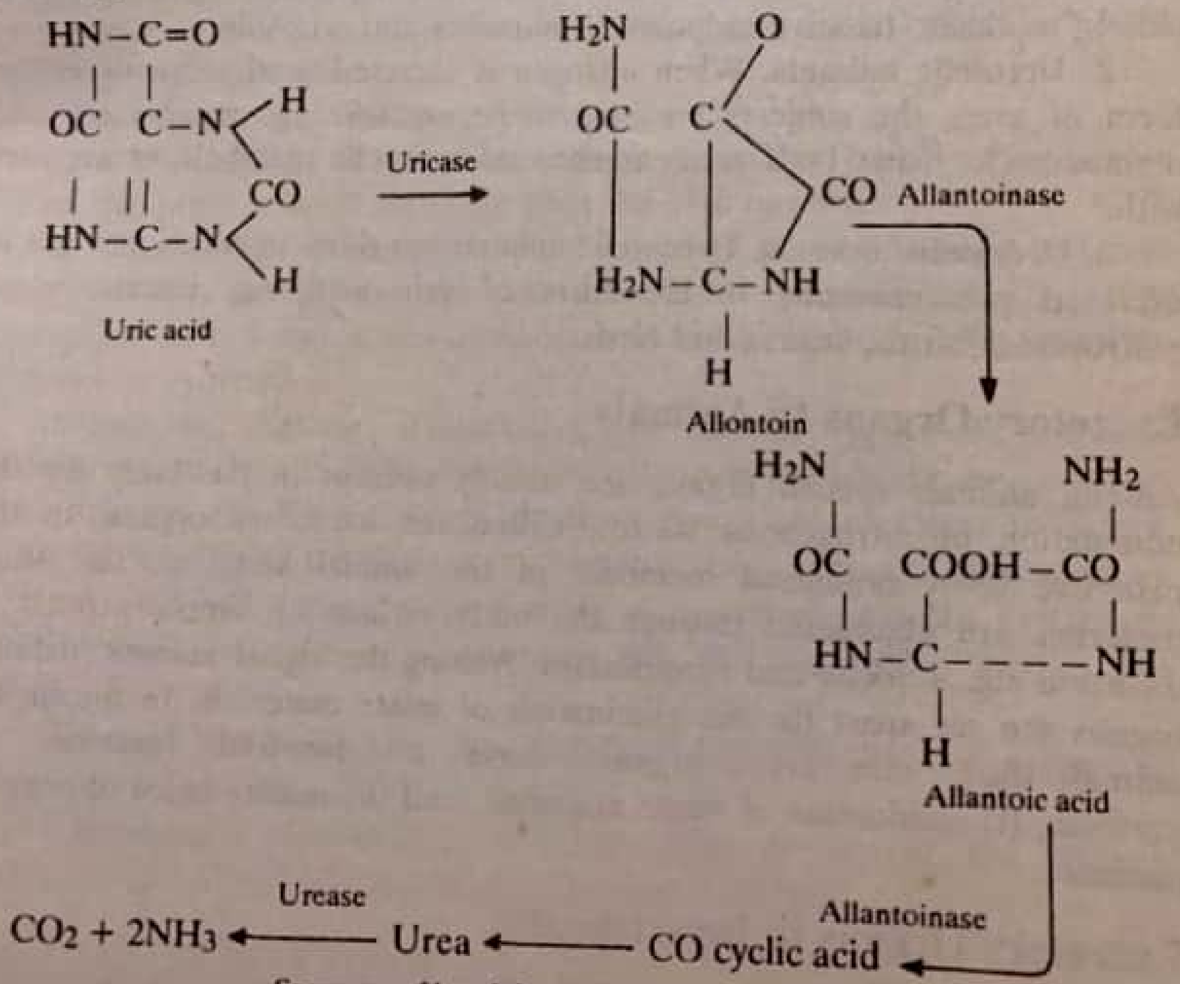
6. Guanine. This nitrogenous excretory product is met among the spiders only. Its solubility is very low and the mode of its formation is not known.

7. Purine. The purines are degraded to different degrees by animals of various groups. Some animals excrete a high proportion of purines without further degradation. In some animals, adenine may be oxidized but not in others.

8. Allantoin. This is formed from uric acid as a result of an oxidation reaction catalysed by an enzyme uricase and may be excreted in many forms.

9. Allantoic acid. Further oxidation of allantoin by allantoinase enzyme results in the formation of allantoic acid.

10. Hippuric acid. This acid is found in mammals. The benzoic acid present in the food of mammals is removed and it combines with glycine to form hippuric acid.



Sequence of breakdown of uric acid into NH_3

11. Orinithinic acid. This acid is formed in birds as a nitrogenous decomposition product. The benzoic acid present in the food combines with ornithine to form ornithinic acid.

12. Creatine. Isotope experiments have shown that creatine is synthesized in the liver from three amino acids, viz., arginine, glycine and methionine. Creatine is liberated into the blood and is taken up by the muscles when required. The normal level of creatine is 2 to 8 milligrams/100 ml of blood. The excess of creatine is excreted along with urine.

13. Creatinine. It is formed in the body from creatine through creatine phosphate. The normal level of creatinine in the blood is said to be about 1 mg 100 ml.The extra amount of the creatinine is to be excreted with the urine.

Classification of animals based on nitrogenous excretion

Animals are broadly classified into three groups, based upon the type of nitrogen excretion.

1. Ammonotelic animals. Animals are described as being *ammonotelic* when nitrogen is excreted predominantly in the form of NH_3. This is especially true in most of the aquatic animals i.e. certain protozoans, actinozoans, polychaete annelids, crustaceans, *Aplyisa, Sepia, Octopus,* among molluscs, teleosts, tadpoles of amphibia and crocodis.

2. Ureotelic animals. When nitrogen is excreted predominantly in the form of urea, the subjects are said to be *ureotelic* e.g. amphibians and mammals. In fishes both ammonotelic and ureotelic metabolisms are met with.

3. Urecotelic animals. Urecotelic animals are those in which nitrogen is excreted predominantly in the form of uric acid e.g. insects, some gastropods, lizards, snakes and birds.

Excretory Organs In Animals

Among animals special organs are usually present in the body for the elimination of nitrogenous wastes, called the excretory organs. In the primitive lowly organised members of the animal kingdom, the waste meterials are eliminated through the body surface by simple process of diffusion e.g. *Amoeba* and *Paramecium*. Among the higher animals, definite organs are set apart for the elimination of waste materials. In the higher animals the excretory organs have a two-fold function to perform, (i) elimination of waste materials, and (ii) maintenance of osmotic pressure.

Excretory Organs in Invertebrates

Protozoa. The contractile vacuole is often credited with the function of removing the waste products. The old view, that the contractile vacuole is excretory in nature, is not accepted in the present day. Nowadays it is looked upon as an osmoregulatory organelle. Its function is to remove the excess of water that has entered into the protoplasm by endosmosis. Excretion is effected by simple diffusion through the body surface.

Porifera and Coelenterata. In these animals excretion is effected by direct diffusion from the body cells into the external medium.

Platyhelminthes. A fairly well-organized excretory system is present among the flatworms. Morphologically the fundamental unit of the excretory system is the *flame cell*.

Nematoda. Among the parasitic nematodes the excretory system is represented in the form of longitudinal tubes with or without flame cells. Cob cells may be present below the oesophagus which are usually flask-shaped or pyriform structures.

Annelida. Nephridia are the excretory organs of annelids. Nephridia occurs in many varieties which can broadly be classified into two types :

(a) Protonephridium, (b) Metanephridium.

(a) Protonephridium. It contains solenocytes and in this feature the excretory system of annelids resembles that of the flatworms.

(b) Metanephridium. Metanephridia find their truest expression among the terrestrial annelids e.g. *Lumbricus*. In *Megascolex*, for example, there are three types of nephridia viz. :

(i) Meganephridia, which are typical metanephridia.

(ii) Micronephridia or *integumental nephridia*, whose number varies from 100 to 150, present in all segments from the 14th onwards.

(iii) Pharyngeal nephridia or *Typical nephridia* present in segments 5 to 9 at the sides of oesophagus and gizzard. In addition certain cells, known as *chloragogen cells*, found in the coelomic wall of the worm are also regarded as excretory in function.

Arthropoda. Among crustaceans two distinct types of excretory structures are met with. In Malacostraca antennary glands are the excretory organs. Among the Entomostraca maxillary glands are excretory in nature. In insects Malpighian tubules are the excretory organs. In the Onychophora there are 14 to 42 nephridia functioning as excretory units. In Myriapods and Arachnids, the excretory structures are the Malpighian tubules and book-lungs.

Mollusca. The nephridia are usually thought to be the organs of excretion.

Echinodermata. Specialized excretory organs are absent. Excretion is effected by the amoebocytes found in the coelomic fluid.

Excretory organs in vertebrates

Kidneys are the chief excretory organs of the vertebrates. The adult kindney is composed of a number of structural and functional units, known as *nephrons*. The individual nephron bears a striking resemblance in structure as well as in physiology to a nephridium of the invertebrates. There are three types of vertebrate kidneys, depending upon their position and state of development in the life history. They are named by British embryologist Francis Balfour as *pronephros, mesonephros* and *metanephros*.

1. Pronephros. It is the an embryonic structure in all vertebrates except *Bdellostoma* and *Myxine,* where it is the functional excretory organ in the adult.

2. Mesonephros. It is the functional kidney of Petromyzon, fishes and amphibians. It is the functional excretory organs during the embryonic stage of reptiles, birds and mammals.

3. Metanephros. It is the functional kidney in the reptiles, birds and mammals in the adult stage.

Structure and Function of Typical Kidney

There are two kidneys which are dark red, bean-shaped, placed one on either side of median vertebral column in the lumbar region, but not on the

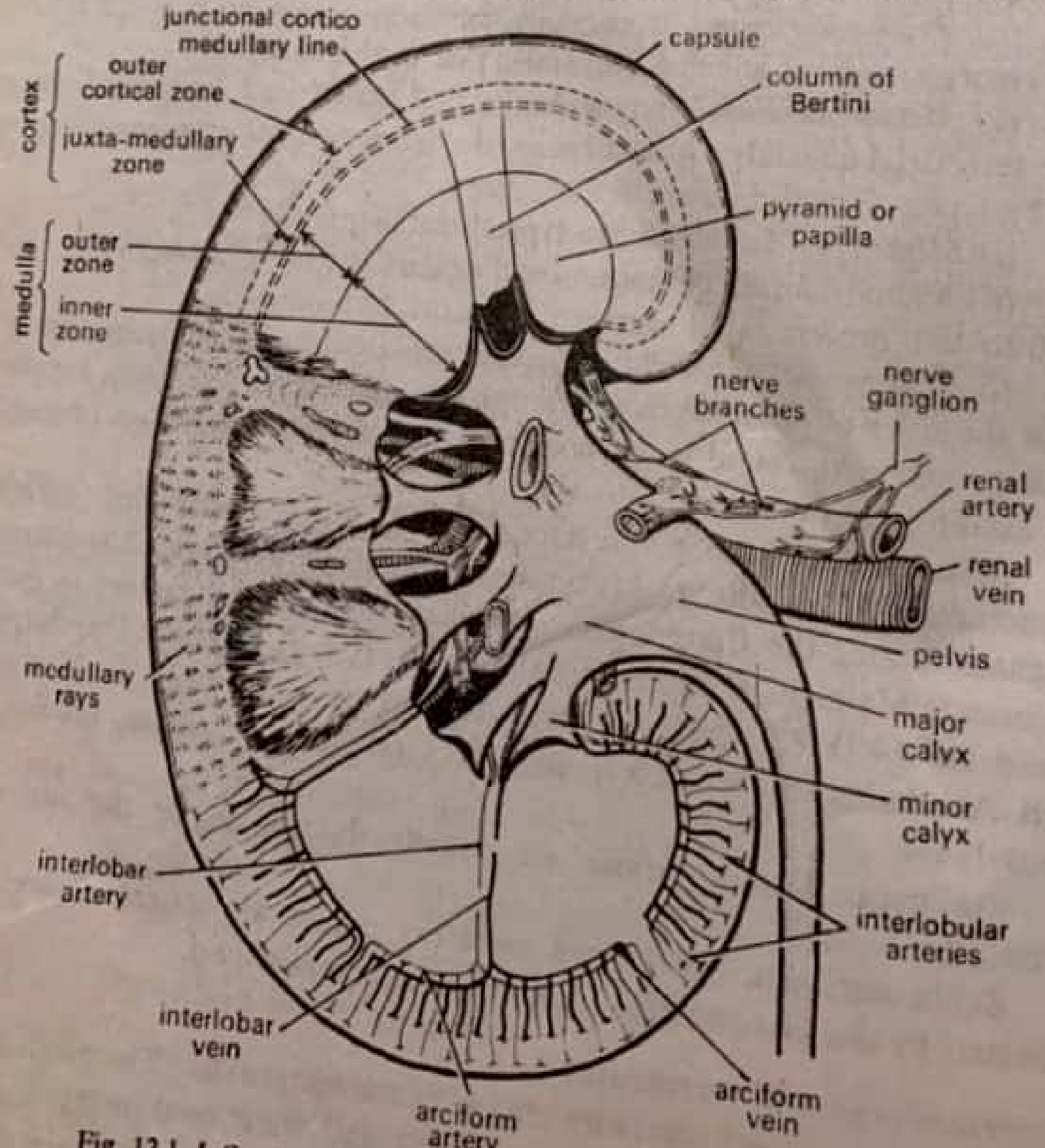


Fig. 13.1. L.S. of mammalian kidney to show internal structure.

same level. In the case of man, right kidney is slightly on the lower side due to the fact that the right side of the abdominal cavity is occupied by the liver. Outer surface of each kidney is convex and inner concave which faces the vertebral column. A depression called *hilus* is present on the concave side, from where ureter takes its origin and joins the urinary bladder backwardly. The renal artery and renal vein pass in and out at the hilus.

Internal structure of kidney. Internally each kidney is distinguished into two zones—an outer dark red zone, *cortex* and inner pale red zone, the *medulla.* Ureter entering through the hilus expands, forming a wide funnel-shaped structure, *pelvis*, which has at its free end a number of cup-like cavities called *calyces* and each cavity is called as *calyx.* Medulla is distinguished into cone-like structures, called renal *pyramids* (Fig. 13.1).

Microstructure of the kidney. Each kidney contains a number (million) of *nephrons,* which are referred as the structural and functional units of the kidney. Nephrons are concerned with the separation of urine from the blood.

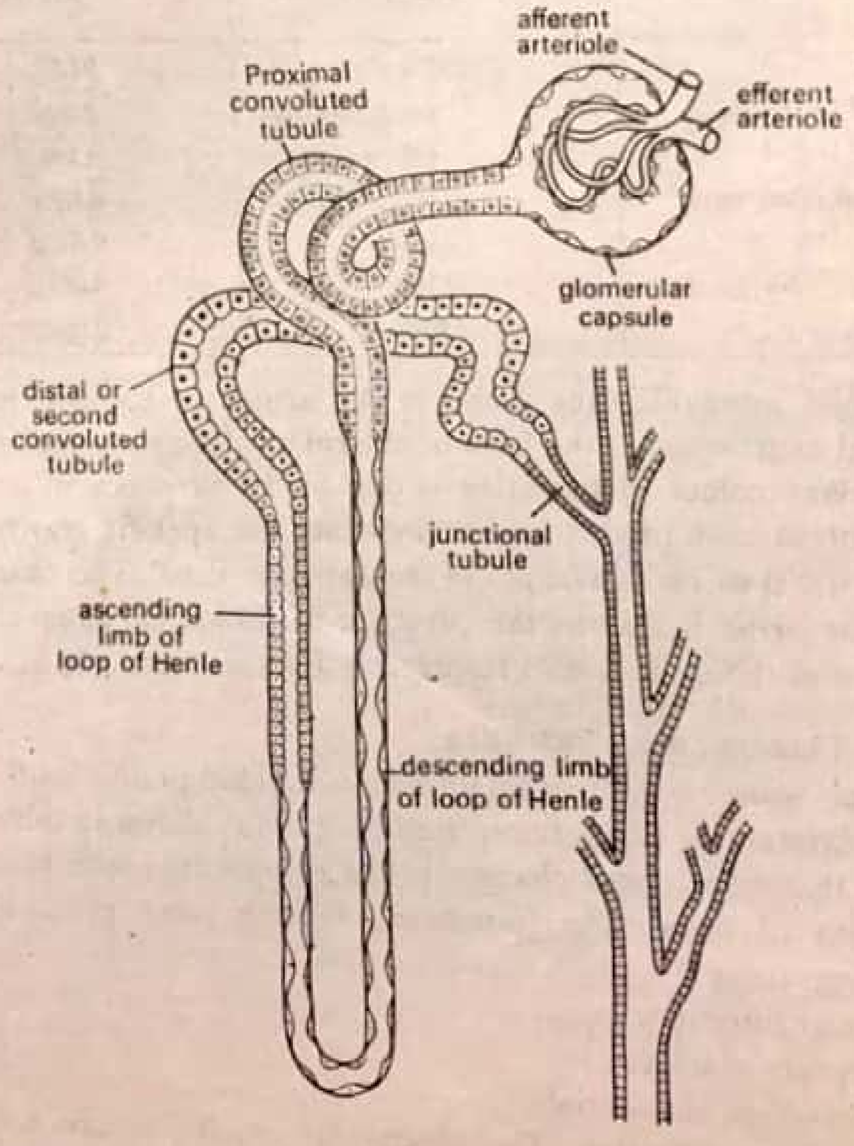


Fig. 13.2. A nephron with collecting tubule of the mammalian kidney.

Nephron begins with *Malpighian capsule* and is followed by a very much coiled *uriniferous tubule*. The former consists of *Bowman's capsule* and network of blood capillaries and referred as *glomerulus*. Malpighian Tubule is followed by a short neck which is followed by a coiled portion, the first "U"- shaped convoluted tubule, the *Henle's loop*, which has two loops i.e. *descending* and *ascending limbs* (Fig13.2). The ascending limb of Henle's loop again gives coiled structure, the *second convoluted* tubule opening into *collecting tubule* and finally into larger tubule, known as *duct of Bellini*, which in turn opens into the pelvis of ureter at the apex of renal pyramids.

Urine. The chemical composition of urine shows it to be quite different from blood plasma as shown in Table 13.1.

Table 13.1 **Showing relative concentration of some of the components of urine and blood plasma**

Component	Concentration	
	Urine	Plasma
Water	97%	91.5%
Protein	0% (normally)	8.0%
Glucose	0% (normally)	0.1%
NaCl and other salts	0.6%	0.85%
Urea	2.0%	0.03%
Creatine	0.1%	0.001%
Uric acid	0.05%	0.004%

The chief inorganic salts found in the urine are sodium, potassium, calcium and magnesium in the form of chlorides, phosphates and sulphates.

The yellow colour of the urine is due to the presence of *urochrome*, which is a breakdown product of haemoglobin. The specific gravity of urine varies from 1.003 to 1.030. The pH of normal urine is 6.0. The characteristic odour of the urine is due to the presence of *urinod*. A little amount of NH_3 present in the urine is also responsible for the odour of the urine.

Physiology of kidney : urine formation

Since the only source for the urine formation is blood plasma and since the urine is so different in composition from plasma (as shown in table 13.1), it is apparent that some major changes in the composition take place during the formation of urine. The formation of urine takes place along the following three steps :

(1) Glomerular filtration.

(2) Reabsorption of solutes.

(3) Active excretion of materials.

1. Glomerular filtration. The glomerular capilla-ies are not simple loops but form a freely branching network. This type of arrangement produces skimming of the plasma relatively freed of cells into the small capillaries where actual filtration takes place. The blood cells flow directly into the efferent arterioles. This type of arrangement reduces the rate of flow of blood and turbulence thus facilitating the filtration process.

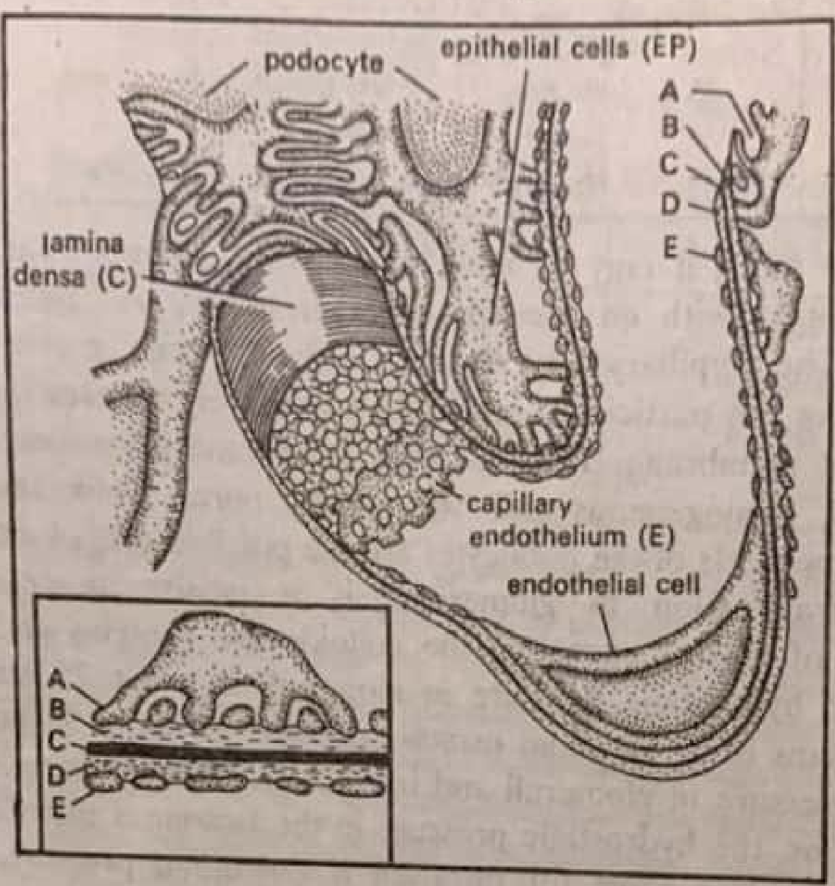


Fig. 13.3. Fine structure of glomerular capillary and related parts of Bowman's capsule. A—Visceral layer of capsule formed by podocytes. B & D—Cement layers. C—Lamina densa. B, C & D form basement membrane. E—Capillary endothelium. EP—Epithelial cells.

Electron microscopic studies have revealed that the capillary endothelium (Fig. 13.3) called the *lamina fenestrae* has pores of 400-900 Å in diameter. These pores are too large for ultrafiltration but they expose the ultrafiltration membrane, the *glomerular basement membrane* (0.1 μm thick) to the free flow of plasma by removing the endothelial cytoplasmic barrier. This layer is a homogeneous layer and acts as a limiting membrane for restraint of plasma membrane. The visceral layer of the Bowman's capsule is made of a large number of specialized cells, the *podocytes* which possess numerous foot processes (*pedicels*) resting on the basement membrane. The spaces between the foot processes are very narrow (100 Å) and are known as the *slit pores* are the sites of ultrafiltration of plasma proteins. As shown in the table below, plama proteins beyond a particular size are retained in the plasma.

Transport of glucose is also dependent on the intratubular Na^+ concentration.

Reabsorption of protein. Though protein is filtered out in the glomeruls, a small amount of it escapes into the ultrafiltrate. Protein in the fluid is reabsorbed by pinocytosis. The plasma membrane engulfs protein and forms pinocytotic vesicles. The protein in the vesicles is digested by lysosomal enzymes and the liberated aminoacids are evenly distributed.

Table 13.3. **Showing forces producing ultrafiltration in the Malpighian body.**

Pressure	Glomerular Capillary Blood	Bowman's Capsule
Hydrostatic pressure	60 mm Hg	10 mm Hg
Colloid osmotic pressure	25 mm Hg	0 mm Hg
Effective filtration pressure	35 mm Hg	10 mm Hg
Actual effective filtration pressure	25 mm Hg	

2. Reabsorption of solutes. If the glomerular filtrate composition is similar to the nonprotein portion of blood, it is obvious that much of the water and dissolved substances must be returned to the blood before the urine as such is eliminated. It is also evident by the comparison of relative concentration of some of the components of urine and plasma (Table 13-1). *Reabsorption* takes place along the renal tubules. The glomeruli (both kidneys) filter 170 liters of solution per day in normal man. Since the volume of urine excreted in a day is about 1.5 liters, 99 per cent of the glomerular filtrate is reabsorbed.

Some 80 to 87% is reabsorbed along the upper (proximal) portion of the tubule, this fraction is referred to as *obligatory reabsorption*. Along this section of the tubule the urine remains isosmotic with blood plasma. Concentration of the urine takes place along the lower (distal) portion of the tubule; this is known as *facultative absorption*. This fraction amounts to 13 to 20 per cent of the total resorption. It is reabsorbed against the osmotic pressure of urine, and it requires energy which is provided by the metabolic activity of the renal tubular cells.

3. Active excretion of materials. The active excretion of materials involves the diffusion of materials from the capillary beds into the kidney tissue fluid and then in metabolic transfer from the tissue fluid into the lumen of tubules. Potassium and hydrogen ions are materials which are actively excreted in this manner.

Ammonia and hippuric acid are formed in the kidney and actively excreted. This formation of ammonia will prove to be important in the maintenance of the acid-base balance, since the ammonia formed can take the place of sodium, allowing sodium to be reabsorbed and conserved as base.

Table 13.2. Sizes of proteins and their ability to filter through glomerulus.

Proteins excreted	Diameter Å	Mol Wt.	Proteins not excreted	Diameter Å	Mol. Wt.
Gelatin		35,000	Serum albumin	150	69,000
Egg albumin	88	43,500	Serum globulin	–	103,800
Myoglobin	54	17,000	Edestin	–	208,000
Haemoglobin	54	68,000	Haemocyanin	–	5,000,000

From the table, it may be noted that serum albumin is at the critical level of filtration with an average diameter of 150 Å based on X-ray diffraction. The capillary endothelium with its large pores (900 Å in diameter) does not participate in filtration but freely exposes the plasma to the basement membrane. The basement membrane is considered to be a hydrated gel, homogeneous and without any pores. Thus, the slit pores between the pedicels of the podocytes are the possible sites of ultrafiltration.

The ultrafiltration in glomerulus is a passive process. All the constituents of plasma excepting the colloids like proteins and lipids are filtered. The hydrostatic pressure in glomeruli is about 75 mm Hg. The plasma proteins exert a colloid osmotic pressure of about 30 mm Hg. The interstitial pressure in glomeruli and intratubular fluid pressure are 10 mm Hg each. Thus, the hydrostatic pressure in the Bowmnas capsule is 20 mm Hg. The motivating force for filtration is the blood pressure, while the colloid osmotic pressure and hydrostatic pressure in the Bowman's capsule oppose the filtration process. The *effective filtration pressure* is about 25 mm Hg $(75 - 30 + 20)$. The filtration process stops if the pressure in the capsular space is raised to the level of effective filtration pressure. Intravenous injection of saline reduces colloid osmotic pressure of plasma proteins and increases glomerular filtration rate. Micropuncture of the Bowman's capsule and analysis of the fluid in the capsular space collected with the help of a micropipette show that the composition of the fluid is similar to plasma except for the absence of plasma proteins.

Reabsorption of glucose. Glucose is completely reabsorbed in the first half portion of the proximal tubule. Increase in the concentration of glucose in plasma increases the amount of glucose reabsorbed. Absorption of glucose is against concentration gradient and is carrier mediated. The rate of reabsorption of glucose is therefore dependent on the concentration of the carrier protein in the cytoplasm of tubular cells and the availability of these molecules. The *transport maximum*, Tm, is the maximum amount of a substance that can be transported by tubular cells in a given time period. It is also dependent on the number of carrier sites for the substance present in the carrier protein molecule and the amounts of the required enzymes present. In human beings the Tm for glucose (Tm. G) is about 320 mg/min.

movement of NaCl help to maintain the osmotic concentration gradient of the interstitial fluid in the medulla. Very little quantity of NaCl is carried away by the blood and the osmotic concentration of the blood that enters and leaves the vasa recta is the same. Blood flow through the vasa recta is very slow and it becomes still slower as the concentration becomes increased in the medullary region.

Production of concentrated urine : counter current theory

The production of concentrated hypertonic urine is a complex process and is closely related with the distribution of the ranal tubules and the concentration of Na^+ in the interstitial fluid at different levels of the cortex and medulla of the kidney (Fig. 13.4).

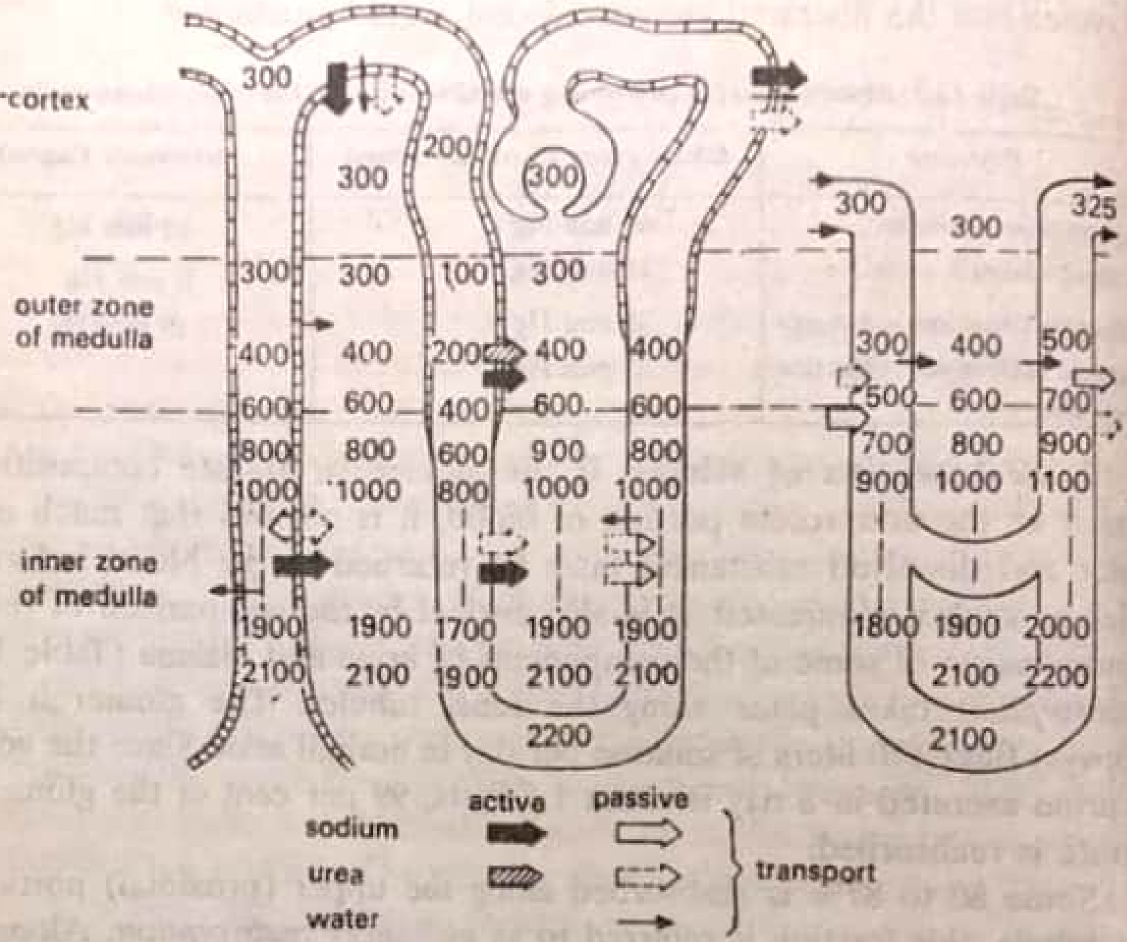


Fig. 13.4. Diagram illustrating the countercurrent mechanism as it is believed to operate in a nephron with a long loop in the vasa reacta (represented on the right). The numbers represent hypothetical osmolality values. No quantitative significance is to be attached to the number of arrows and only net movements are indicated.

The loop of Henle with its surrounding blood vessels descends from the cortex into the medulla and after taking a turn again enters the cortex. This arrangement of the loop resembles that of a hairpin. The osmotic concentration of the peritubular fluid increases towards the medulla. The glomerular filtrate has an osmotic concentration equal to that of plasma. In the proximal tubule about 80% of fluid is reabsorbed. Thus, the fluid entering the descending limb of the loop of Henle is *isotonic* and the surrounding interstitial or peritubular fluid is *hypertonic*. Therefore, sodium ions passively diffuse into the tubular fluid and make it *hypertonic*. During the passage of fluid through the ascending limb of loop of Henle, sodium ions leave the tubular fluid and reenter the peritubular fluid. The ascending limb is impermeable to water. Thus, the fluid in the tubule becomes *hypotonic* as it enters into the distal convoluted tubule.

increase in arterial pressure.

Control of Kidney Functioning

The main function of kidney is excretion i.e. the production of urine. This occurs by the co-ordinated activity of the different fundamental units of kidney called nephrons. The processes are filtration by Bowman's capsule and reabsorption and secretion by the urinary tubules. If this process is changed due to any condition the urine formation will also alter.

The process of glomerular filtration is dependent on the blood pressure in the glomeruli. If blood pressure is low it reduces the rate of glomerular filtration and also decreases the rate of urine formation. On the other hand rise of blood pressure in glomeruli by injection of adrenaline or nor adrenaline will increase the glomerular filtration rate and also increases the rate of urine formation.

The processes of reabsorption and secretion are more or less active and involve energy utilization in the form of ATP. Some factors like low temperature, metabolic poisons like cyanide and dinitro phenol will decrease or stop the tubular reabsorption and secretion. So the formation of urine will be dilute or more in volume.

There are certain other factors also which control the rate of urine formation. Some hormones like aldosterone from adrenal cortex and antiduretic hormone from posterior pituitory also control the rate of urine formation.

Metabolism of Nitrogen

Principal source of N_2 is protein of the food and in all the animals the liberation of ammonia from the proteins takes place as follows :

$$\text{Proteins} \xrightarrow[\text{enzyme}]{\text{Proteolytic}} \text{Amino acids} \xrightarrow[\text{(in liver)}]{\text{Deamination}} \text{Ammonia}$$

As mentioned earlier animal are called as *Ammonotelic*, *Ureotelic* or *Urecotelic*, depending upon the type of nitrogenous excretory materials excreted.

Ammonotelic animals

Animlas are described as being ammonotelic when nitrogen is excreted predominantly in the form of ammonia. It is especially true in most of aquatic animals i.e. certain protozoans, actinozoans, polychaetes, annelids, *Aplysia*, *Sepia*, *Octopus* among molluscs, teleosts, tadpoles and amphibia.

In ammonotelic animals NH_3 is the chief excretory product. In general, aquatic invertebrates excrete nitrogen in the form of ammonia. This is because the solubility of NH_3 is very high and it can readily diffuse out into the surrounding water medium through the body surface. The toxic or the poisonous nature of NH_3 can be exemplified by the following points.

(1) Sumner showed that, if crystalline urease is injected into reptiles, NH_3 is formed by hydrolyzing small amount of urea present in the blood and if the concentration of urease reaches above one part in 20,000, death results. Thus, ammonia requires plenty of water for its excretion and since it is extremely toxic it must be removed rapidly and efficiently from the body. This probably accounts for the occurrence of NH_3 as the main excretory porduct in the aquatic animals. The amount of NH_3 liberated varies widely according to diet—(i) In *Paramecium* 91% of excreted nitrogen is ammonia when they had been fed on glycine, (ii) no ammonia had been liberated when they were fed on starch, and (iii) in one urea, uric acid, creatinine on whole protein diet (was found).

(2) *Spirostomum* produces a large quantity of ammonia when the oxygen tension is less than that of the air.

(3) Various echinoderms—starfish, sea urchins and sea cucumbers are ammonotelic but they also excrete considerable amount of amimo acids into the water.

(4) Among arthropods, NH_3 seems to predominate as an excretory product in the fresh water as well as marine crustaceans, though they are also said to liberate amino acids into the water.

(5) Among molluscs the cephalopods—*Sepia* and *Octopus*—excrete most of their nitrogen as NH_3. The pelecypods which have been examined, both in marine and fresh water (except *Mytilus*), are also ammonotelic.

The permeability to water of the distal convoluted tubular epithelium is under the control of the *antidiuretic hormone*. Under the influence of this hormone, water is absorbed by the distal tubule resulting is an isotonic state of the fluid. As this isotonic fluid passes through the collecting tubule, it becomes again hypertonic due to the ionic and water exchange between the tubular fluid and in the interstitial fluid of medulla. Thus, finally, a hypertonic urine is produced in the kidney.

Sometimes, when body fluids are diluted, dilute urine has to be excreted by animals. The volume of water osmotically transported from the collecting tubule into the peritubular fluids depends on the permeability of the collecting tubule. The permeability of collecting tubule is controlled by antidiuretic hormone or vasopressin. In the absence of vasorpressin the epithelium of the collecting tubule is impermeable to water, and water cannot leave the tubules in respsonse to the higher osmotic concentration outside. Under these conditions, a dilute urine is produced and excess water is excreted. When water conservation becomes necessary, the hypothalamo-hypophyseal system is activated and vasopressin is released. The collecting tubular epithelial cells permeability to water is increased, and water is absorbed resulting in concentrated urine production.

The peritubular fluid remains hypertonic due to (i) active transport of one or more solutes by the ascending loop of Henle, (ii) differences in the permeability to NaCl and water in the ascending and descending limbs of the loop of Henle, and (iii) the 'U' shaped configuration of the loop of Henle resulting in a counter current of the tubular fluid. The ascending limb actively transports Na^+ and Cl^- but impermeable to water. The descending tubular cells are permeable to water and passive diffusion of NaCl. Thus, the descending and ascending limbs constitute a counter current flow system and the active and passive movements of NaCl establish an osmotic gradient in the interstitial fluid. As extremely high concentrations of NaCl can be produced at the tip of the loop of Henle in the medulla, the system is known as *counter current multiplier system*.

Vasa recta. The concentration of NaCl can also increase in the interstitial fluid of the medulla because of the counter current flow system of the vasa recta. Some of the peritubular capillaries arising from the efferent arteriole descend into the medulla along with the loops of Henle. The osmotic concentration of the blood in the descending limb of vasa recta is about 320 m osm. During the passage of the tubule deep into the medulla, NaCl from the medullary fluid enters the flowing blood of the vasa recta through the very permeable walls. Thus, the osmotic concentration of the blood goes on increasing towards the top of the vasa recta. As the blood takes a turn in loop and flows upward in the ascending limb into the more dilute region of the medullary peritubular fluid, NaCl leaves the blood and diffuses into the medullary fluid. The counter current in the vasa recta and

Among gastropods *Aplysia* is ammonotelic but excretroy products in other gastropods are diverse.

(6) Earth worms are intermediate between aquatic and terrestrial. This can be illustrated by the following experiment : (i) Earth worms kept under moist air produce more urea than ammonia (Bahl, 1945). (ii) If they are immersed in water they become ammonotelic (Delaunay, 1934). Besides, in the starved earthworms urea constitutes less than 10% in the urine, whereas after starvation urea becomes as much as 86% of the nitrogenous excretion. It would be of great interest to note the effect of H_2O supply on the urea and ammonia ratio in the urine.

(7) Most of the fresh water fishes are ammonotelic though they excrete some urea also. Most of their nitrogenous excreta diffuses out through the gill surface. Many marine teleosts are knwon to excrete considerable amount of NH_3 and a small quantity of urea. Some marine teleosts especially those forms whose blood is hypotonic to seawater excrete nitrogen in the form of trimethylamine oxide. It is a soluble and non-toxic substance.

(8) Nitrogen excretion in lung-fishes has been studied by Smith e.g., *Protopterus.* (i) When these fishes are active in water, they excrete nearly three times more nitrogen as NH_3 than as urea (ii) When aestivated in mud cocoon, however, it excretes urea, which may accumulate to the extent of 1-2% of the body weight in a year. At this time muscle urea content increases by some seven times. (iii) When the fish returns to water, urea is rapidly excreted and excretion of nitrogen as NH_3 increases to its characteristic high proportion.

Since removal of CO_2 and H_2O is effected by respiratory areas of the body surface, excretion becomes restricted to elimination of nitrogenous waste. Nitrogenous wastes take the form of NH_3 chiefly but certain percentage of amino acids, urea, uric acid, purines and other compounds is also excreted. Most aquatic invertebrates, for example, excrete 52.2% of their nitrogenous waste as NH_3, 14% amino acid and the balance is made up of the rest. Since the major fraction of the waste is ammonia these animals are described as ammonotelic. The large quantity of NH_3 produced diffuses out into the surrounding water slowly through the body surface or through other permeable membranes. This diffusion being slow, the tissues of these lowly-organized animals have to tolerate the presence of this poison to the extent of 3% or even to 4.8% (as in snails), while the higher animals, like the rabbit, will die, if even 0.005% of NH_3 remains in the blood.

(9). In most ammonotelic animals, the NH_3 is flushed out of the body by water either through the body surface or the gills.

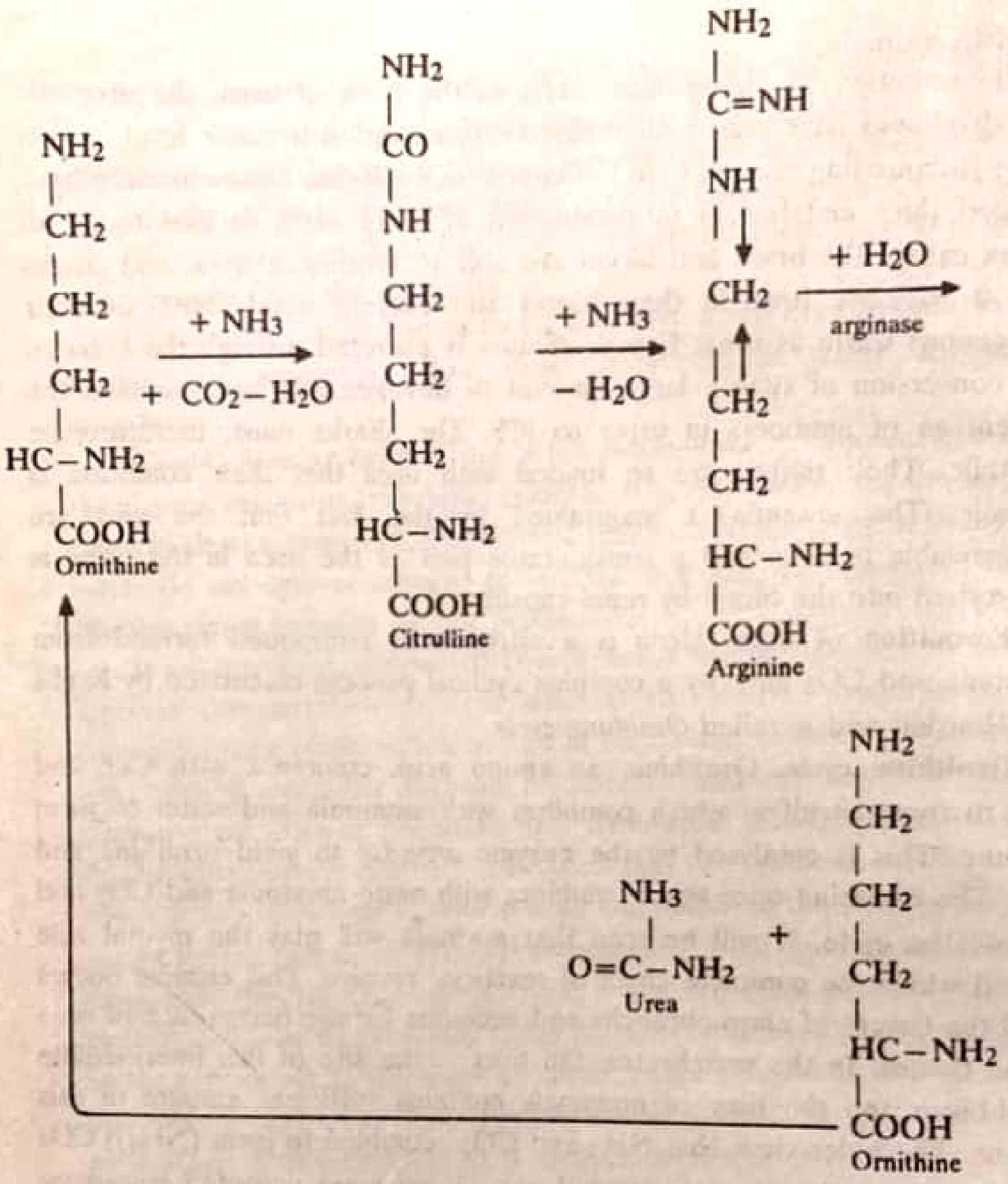
(10) Marine and fresh water fishes, *Limnaea,* starfishes, *Carcinus, Lumbricus* and the other lower invertebrates excrete definitely large percentage of NH_3, and since these live in the aquatic medium, they have plenty of water to flush the ammonia.

Earthworms which live in moist soil have more urea in their urine and excrete less ammonia, while those immersed in water are definitely ammonotelic.

Trimethylamine oxide. This is the nitrogenous compound occurring in the tissues and urine of marine teleosts. It is responsible for the unpleasant smell of dead marine fish. The closest relative of these teleosts living in fresh water do not appear capable to synthesize it and their urine is free of it. Marine elasmobranchs do not produce it.

It is, therefore, suspected that it helps marine teleosts to raise their internal concentration as urea does in the case of elasmobranchs. But trimethylamine oxide which occurs in blood and tissues only in very small quantities is diffusible through membranes and any dissolved substances cannot exert osmotic presssure at a membrane through which it can pass freely. Therefore, it must be concluded that the synthesis of this substance, which is less toxic than ammonia is an adaptation to the shortage of H_2O, so necessary for the quick removal of ammonia from the tissues. Biochemists think that this trimethylamine oxide is formed from protein residues like choline obtained by the break down of nitrogenous fats (lipoproteins). Small traces of it occur in the urine of other animals like cephalopods where the pathway of degradation of lipoprotein is similar. In other words, large percentage of this compound is formed in marine teleosts, because of the physiological scarcity of H_2O in marine teleosts. In spite of 28% of the total nitrogenous waste being excreted in the form of this compound, marine teleosts are ammonotelic because 56% of nitrogen excreted is in the form of ammonia.

Thus, ammonotelism is a feature related to the habitat and mode of life of the animal and is illustrated by lung-fish. When there is plenty of water, it excretes nitrogen three times more in the form of NH_3 than as urea, but during draught condition, when it aestivates in the mud, it excretes large amount of urea, and shows a seven-fold increase of muscle urea. Ammonotelic metabolism is thus conditioned by the supply of water.



Reactions involved in Kreb's ornithine cycle.

Amphibia. The livers of both frogs and toads can synthesize urea and the cells of their renal tubules appear capable of excreting urea.

The tadpole is ammonotelic like the fishes, over 40% of their nitrogenous excretion being in the form of NH_3.

As the tadpole metamorphoses into the adult, ammonia decreases to 12.5% and urea increases proportonately. This evidently is an adaptation to life on land, where the adult frog cannot have as much water as the tadpoles get. Urea is much diffusible and soluble substance like NH_3, but differs from it, in that it is less toxic even in relatively high concentration and hence can be retained in the body for some time before being eliminated.

Ureotelic animals

By the retention of nitrogenous waste in the form of urea, the ureotelic animals should have raised their blood concentration to same level as that of the surrounding seawater. In the course of evolution elasmobranchs have changed thier metabolism to production of more urea, so that today all tissues except the brain and blood are able to synthesize urea, and sharks have 2 to 2.5% urea in their blood and excrete neraly 80% of their nitrogenous waste as urea. Excess of urea is excreted through the kidneys. The conversion of such a large amount of nitrogen into urea reduces the percentage of ammonia in urine to 8%. The sharks must, therefore, be ureotelic. Their tissues are so loaded with urea that their condition is uraemic. The uraemia is maintained by the fact that the gills are impermeable to urea and a considerable part of the urea in the urine is reabsorbed into the blood by renal capsules.

Formation of urea. Urea is a nitrogenous compound formed from ammonia and CO_2 and by a complex cyclical process elucidated by Krebs and Hensleit and is called *Ornithine cycle*.

Ornithine cycle. Ornithine, an amino acid, combines with CO_2 and NH_3 to form citrulline which combines with ammonia and water to form arginine. This is catalysed by the enzyme *arginase* to yield ornithine and urea. The ornithine once again combines with more ammonia and CO_2 and repeats the cycle. It will be seen that arginase will play the pivotal role around which the complete chain of reactions revolve. This enzyme occurs in all the tissues of elasmobranchs and accounts for the occurrence of urea in the tissues. In the vertebrates, the liver is the site of this intermediate metabolism and the liver of mammals contains sufficient amount of this enzyme. The older view, that NH_3 and CO_2 combine to form $(NH_4)_2CO_3$ and the subtraction of water yielded urea, is no more accepted nowadays. Since Kreb's explanation involves the production of ornithine, this cycle is knwon as the *ornithine cylce.*

In birds urea is not formed although ornithine is present in kidneys because of the absence of arginase in liver. As ornithine is used over and over again, it may be considered to play the part of coenzyme of arginase. Since the chief end product of protein metabolism is urea in elasmobranchs, amphibia and mammals, these are described as ureotelic.

how adaptations cannot be so easily reversed, especially if they are genotypic.

5. Other invertebrates. Even in ammonotelic forms, uric acid has been found to occur in the blood or coelomic fluid or in the tissues.

In *Chaetopterus* it amounts to 7.3 mg in 100 ml and in the crayfish 2.3 mg/100 ml of the urine. Since even in the mammals (which are ureotelic) as much uric acid as 0.23 mg/100 ml of urine may occur, the distinction between these and the truly urecotelic animals, like birds, appears to be based on quantitative differences in the amount of uric acid produced through nitrogen metabolism.

Nitrogen metabolism and recapitulation

According to the theory of recapitulation, *"an embryo in the course of its development passes through stages at which it resembles to a greater or lesser extent, the embryos of the animals from which it has evolved."* Side by side with the gradual differentiation of adult organs and systems, the patterns of metabolism also change.

The frog's tadpole resembles the fish not only in its aquatic breathing habit, but also in its ammonotelic metabolism. When it metamorphoses into an adult frog, urea becomes the major constituent of urine.

Nudam has shown how the chick embryo also "recapitulates in a chemical sense." When it is three days old, the amount of NH_3 decreases from 50% of dry weight of embryo to 30% of dry weight of embryo, while urea increases from practically a trace to 50 mg and uric acid begins to appear in the urine. About the fourth day uric acid increases to 1100% mg of dry weight, while urea decreases to 60 mg and ammonia to 15 mg.

The entire series of estimations show that up to fourth day the chick is ammonotelic like any aquatic invertebrate and later becomes ureotelic like the amphibia and about the 11th day the chick is definitely urecotelic and continues to be so, though the urine of the adult hen still contains small percentage of urea and ammonia.

Reviewing, the following conclusion may be drawn. The availability of water supply in both embryonic and adult life of animals appears to determine the form of nitrogenous waste that results from the breakdown of protein.

In the fresh water fish and aquatic invertebrates the chief nitrogenous waste is ammonia which diffuses out of the body.

Marine teleosts, which face a shortage of water owing to osmotic stress, produce trimethylamine oxide as well as ammonia.

In amphibia which avails less water and mammals whose water-balance mechanism is highly perfect, the tissues have enough water to flush out urea, which is less toxic than ammonia and can be retained till it is eliminated out through the kidney.

Formation of uric acid

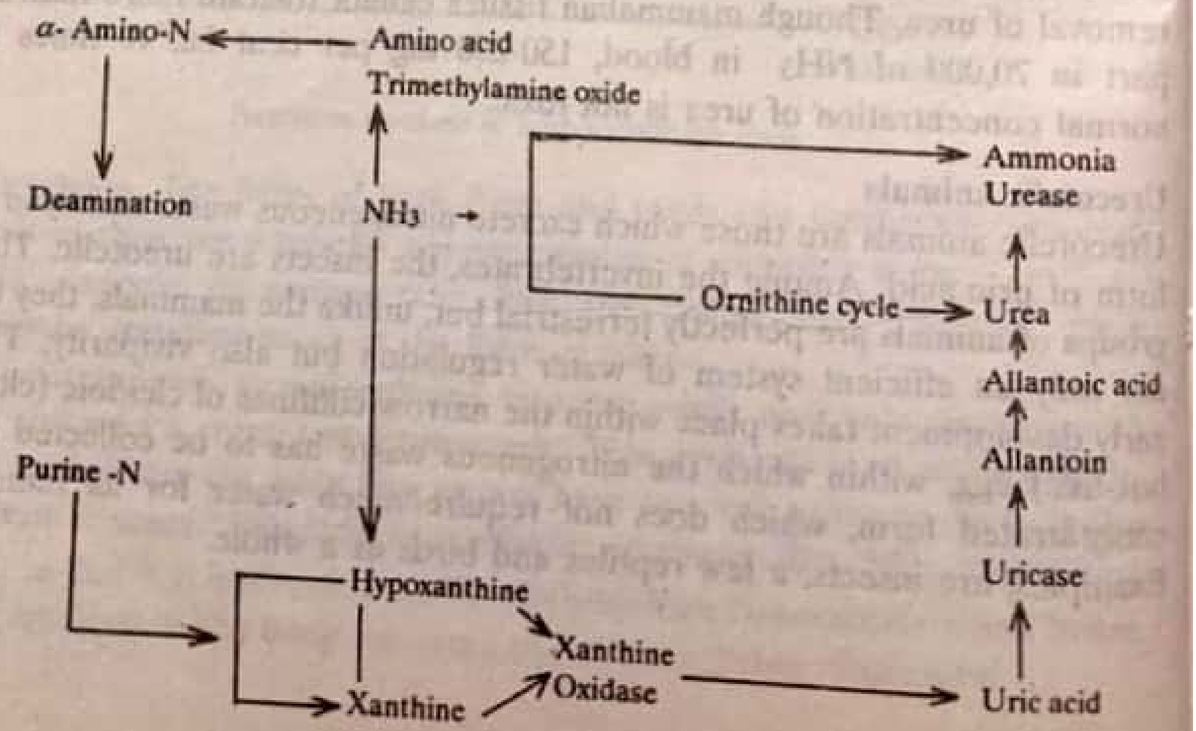
The exact way in which this compound is formed from ammonia has not been experimentally followed but has been made clear from the way in which uric acid can be simplified into ammonia. The following illustrates how ammonia can be related to uric acid as well as to other nitrogenous wastes.

(1) The nucleic acids occur in all the nuclei of the animal body. They are combined with proteins to form nucleoproteins. Hence, apart from the amino acids in the body protoplasm, nuleic acid forms the chief source of nitrogen.

(2) Hydrolysis of nucleic acid yeilds purines and a few pyrimidines. Little is known of the latter. Purines are bases which are excreted as such in several animals.

But reptiles, birds, insects and other animals, which possess the three enzymes, *adenase*, *guanase* and *xanthine oxidase* are able ot convert the purine bases into xanthine and hypoxanthine and oxidize these into uric acid. It is very probable that this is what happens in a large number of urecotelic animals. A small percentage of the uric acid may be excreted with urine, the rest of it is converted into urea by an enzyme uricase and eliminated as urea.

In reptiles, birds and insects, there is no uricase and a large amount of nitrogenous waste is sent out as uric acid. Therefore, these are truly urecotelic animals. They lack arginase as well. Therefore, there is no urea in their urine. While the purine bases from the nucleic acid undergo these changes, the ammonia obtained from the breakdown of the proteins of the cytoplasm as well as surplus of amino acid from food must also be going through such changes as indicated in the following diagram and be reduced to uric acid.



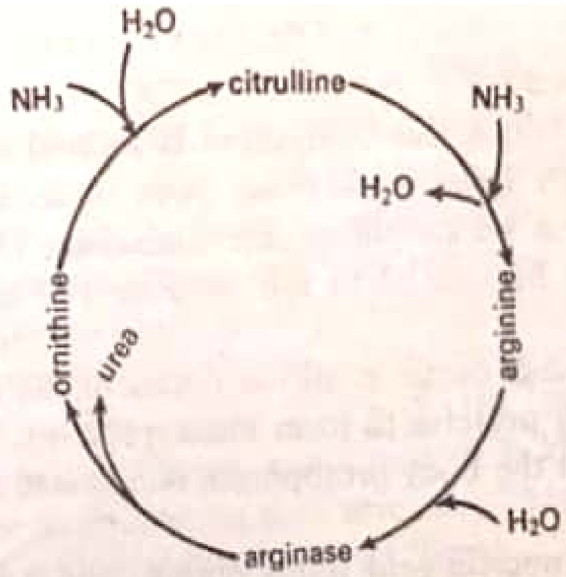


Fig. 13.8. The ornithine cycle (diagrammatic.)

This feature is advantageous to amphibian animals. Production of urea is an adaptation to water shortage, the amphibia had to face on land, just as the elasmobranchs had to face in the marine habitat.

Mammals. Even the most primitve mammals viz. the monotremes are ureotelic. In these truly terrestrial animals, metabolism is ureotelic from the embroyonic period onwards. Being viviparous, they are in connection with the maternal blood stream throughout embryonic life. The water supply is, therefore, as unrestricted as in an aquatic habitat. NH_3 resulting from nitrogen metabolism is detoxicated through formation of urea, which diffuses into maternal kidneys. In the adult, however, closely correlated with the control of the body temperature through the production of sweat and water regulation in general, the body maintains enough water to flush out urea. Further, water is reabsorbed form the urine, and urea in urine is concentrated as much as 100 times, so that not much water is lost in the removal of urea. Though mammalian tissues cannot tolerate more than one part in 20,000 of NH_3 in blood, 150-250 mg per cent i.e. 10 times the normal concentration of urea is not toxic.

Urecotelic animals

Urecotelic animals are those which excrete nitrogeneous waste mainly in the form of uric acid. Among the invertebrates, the insects are ureotelic. These groups of animals are perfectly terrestrial but, unlike the mammals, they lack not only an efficient system of water regulation but also viviparity. Their early development takes place within the narrow confines of cleidoic (closed box-like) egg, within which the nitrogenous waste has to be collected in a concentrated form, which does not require much water for its removal. Examples, are insects, a few reptiles and birds as a whole.

Excretion in some important animal groups

1. Reptiles. Among the modern reptiles, the lizards and snakes live in dry areas, where water conservation is important. Further, their eggs must retain the embryonic nitrogenous wastes till they are hatched. Therefore, their nitrogenous excretion is in the form of relatively insoluble, non-toxic acid, which in house lizards and tree snakes is in the form of solid pellets.

The aquatic reptiles, which have no lack of water, excrete urea. The alligator's urine contains 3.6–6% NH_3, 24.1% urea and 39.1% uric acid.

Chelone mydas excretes urine having 16.1%, NH_3, 38.1% urea and 16.5% uric acid, the balance being undetermined. In the marine turtles urea is proportionately larger in amount than uric acid. This may be due to plenty of water supply rather than indicating ureotelism.

However, unless excretion is studied in several of aquatic chelonia, it is difficult to say wheater it is just an adaptive feature or mere persistence of a more primitive pattern. Whatever primitive feature the morphology and embryology of chelonia may exhibit, such an observation on physiological grounds alone may be misleading.

2. Birds. Birds, as a whole group, are uniform in their physiology. This is very true with their nitrogen excretion. There is about 10% of urea and 3.4% of NH_3 in their urine, indicating the fact that in no animal nitrogenous excretion is in the form of a single product, and that NH_3, urea or amino acid are likely to break through the complex chemical processes of nitrogen metabolism. However, the birds are mainly urecotelic and their faecal matter also includes the solid uric acid.

3. Insects. The bug *Rhodnius* excretes solid urine consisting of 90-92% uric acid in crystaline form with no NH_3 and amino acids, and only a trace of urea. This is true of few insects to whom water supply is too restricted. In several forms the different products vary in amount. *Rhodnius* secretes more urea immediately after a meal of blood, and the blowfly gives off more NH_3 after eating meat. However, it is difficult to be sure if these products are from the gut or are true metabolic wastes, in as much as the Malpighian tubules open into the proctodaeum.

4. Gastropods. Snails, though ammonotelic, have amounts of uric acid varying with the water supply of their environment.

Limnaea lives in water, but lays cleidoic eggs on land and the embryos excrete 4.5% of uric acid. During hibernation the nephridium stores as much uric acid as 3/4 of the weight of the organ.

Terrestrial snails, on the other hand, have larger amount of uric acid in the kidneys of the adults, and those which have secondarily taken to water have relatively more uric acid than the strictly marine forms. From osmotic concentrations one would expect the reverse. But this shows how nitrogen metabolism is closely linked with the possession of requisite enzymes, and

Extreme need for conservation of water is met by the excretion of nitrogen as uric acid in insects, land gastropods, snakes, lizards and birds as well as by the abbreviated development within cleidoic eggs.

The production of urea and also uric acid has arisen independently in several unrelated groups, probably along different pathways of protein degradation, aided by different sets of enzymes. Embryonic recapitulation of biochemical traits of ancestors is indicated in N_2 excretion patterns.

Blowfly larvae are ammonotelic ; tadpoles excrete ammonia in the beginning and they can produce uric acid only after passing through the ammonotelic and ureotelic stages.

Nitrogen excretion appears to have changed so often during the evolution of several groups according to their habitat, that we have to consider the excretion of nitrogenous waste as a labile character.

Among the gastropods, we find NH_3 urea or uric acid being the chief products in different groups. The marine teleosts excrete more urea than the fresh water fish, just as the earthworms excrete more urea then their aquatic relatives. The lung-fishes excrete ammonia when active, but produce urea when aestivate during the dry season. Most of the animals excrete nitrogen in several forms, but only one is the main product. Which product and which enzymatic route is given precedence depends more on the osmotic needs of the animals rather than on their ancestry.

Important Questions

1. Describe the structure and physiology of kidney of a mammal.
2. What is excretion ? Identify the different excretory products met within the different animal groups.
3. What do you understand by ammonotelic, ureotelic and urecotelic animals ? Add a note on the formation of urea in animals.
4. Trace the different pathways along which ammonia is converted into urea and uric acid in different animals.
5. Explain how far the environment of an animal influences excretion of nitrogen.
6. Describe the countercurrent multiplier theory. How is concentrated urine excreted by the kidney ?
7. Describe how kidney functioning is regulated ? What is the role of antidiuretic hormone and aldosterone ?
8. Discuss the role of kidney in acid-base balance and body fluid volume.
9. Write short notes on : (i) Ornithine cycle, (ii) Counter-current theory, (iii) Juxta-glomerular apparatus, (iv) Ultra-filtration
10. What factors determine the rate of ultrafiltration in the glomerulus ?
11. Why is it more adaptive, more animals to reabsorb essential physiological molecules or ions from urine than to secrete wastes actively into the urine ?
12. What evidence is there that the mammalian nephron employs tubular secretion as one means of eliminating substances into the urine ?