Osmoregulation

Introduction

Osmoregulation is a problem faced by all living organisms, even those living in an isosmotic media. In order to prevent destructive osmotic pressures and to maintain a suitable internal osmotic concentration a number of regulatory mechanisms are developed by animals. Hober (1902) first coined the term *osmoregulation to the collective activity of the variety of mechanisms used by organisms to control water movement and water volumes*. This implies the maintenance of an internal osmotic concentration which is different from the surrounding medium. However, it is not sufficient if the water volume is regulated. The internal ionic concentration is also to be regulated as large differences exist in the external ionic concentrations as well as the ionic concentration in the different portions of the body of the animals. The various processes by which the internal ionic concentration of the body are maintained fall under *ionic regulation*. The two processes are inseparable and sometimes the term osmoregulation is used to refer to the two processes.

Study of osmoregulation is a wide field. Various overall mechanisms regulating the internal water and solute levels, the excretory organs, cellular activities basic to these processes and the molecular basis for these mechanisms are all included. Osmoregulation is also closely related to the homeostatic functions like pH and temperature regulation in which body water and ionic concentrations are involved.

If the water content of the body is to be maintained at a constant level, the amount of water entering the body must equal the water which leaves the body. Water enters the animal body in a number of ways—by drinking, along with food, by oxidative reactions in the body resulting in metabolic water or by osmosis from the surrounding medium. The osmosis is caused due to concentration gradients across the semipermeable membrane of the animal. The exit of water also occurs through several routes – through urine, respiration, sweat, through the skin by osmosis and by exocytosis.

The process of osmoregulation is not a passive mechanical equilibrium. It results due to active and negative feed back mechanisms which control ionic and water movements. The basic concepts concerned like the structure of cell membrane, osmosis, diffusion, active transport etc. are first described here.

Nature and role of biological membranes

The chemical composition of the various intracellular media are considerably different from those of the extracellular fluids. Even then, no mixing of the two media occurs and this shows the presence of some barrier between them. This is the *plasma membrane* which allows the passage of selected materials and it is observed that even for the substances permeable, the rate of passage differs greatly.

Biological membranes are not exactly *semipermeable, as the term refers to an ideal membrane which allows the passage of solvent* but not the solutes. Biological membranes are *selectively permeable,* associated with open systems. A number of solutes are impermeable and others permeable. Those which pass through enter at much slower rate than water. Another term preferred is *differently permeable* as the membrane does not select or there is no choice by the membrane. Another important point is that the *membrane is not a passive structural wall. Various membranes differ in their permeabilities to given substances and the permeabilities change in different conditions.* In a number of cases structural changes are found during the entry of different solutes.

Structure. Robertson (1960) developed the *unit membrane* concept of cell membrane. According to this hypothesis biological membranes have a similar, three layered appearance. The outer two dense lines represent the monolayers of protein and the inner less dense zone is the location of a bilayer of lipid molecules. The thickness varies according to the source but usually is about 75 A. The dense layers are 20Å thick and the inner layer is about 35 Å. However, this model of the cell membrane is attacked by several workers (Korn, 1968; Green and Maclennan, 1969) mainly due to uncertainties of where specific electron stains are actually attacking.

Cellular Permeability

Mechanism of movement of material. Solutes and solvent move in and out of the cells by a variety of processes. The most important of them are discussed here.

Osmosis

When two aqueous solutions of different concentrations are placed on either side of a semi-permeable membrane, water passes through it until there is concentration is $\Delta^{-0.63^{\circ}}$, which is less than in sea. This concentration is maintained as in freshwater fishes. Water in large amounts is removed in urine and the salts are reabsorbed. In the gills of these fishes, certain cells can extract the minute amount of salts from fresh water. These animals thus, have a combination of freshwater and marine fishes features. This mechanism is controlled by the endocrine system.

Osmoregulation in terrestrial animals. The osmo and ionic regulation in terrestrial animals is similar to that of marine animals. They are surrounded by a medium which contains a low concentration of water than the body fluids and obtain salts readily through food material. Both these animals have to conserve water and excrete salts.

Conservation of water. Most of the terrestrial animals have a body covering impermeable to water except those living in places where the atmospheric humidity is high. The respiratory surfaces are enclosed within body cavities so that water vapour is conserved and evaporation across the respiratory membranes is reduced. In the insect tracheal system and lungs, the water vapour pressure is very high. In lungs it is 40 mm Hg, while the water vapour pressure in the atmosphere is only less than 1 mm Hg.

In warm-blooded animals the sweat glands secrete water, which withdraw body heat during its evaporation from the body surface. The areas that can secrete sweat are limited and are controlled by the nervous system.

Reabsorption of water. The tubules in the kidneys of terrestrial vertebrates have the capacity of reabsorbing water from the urine. Almost 99% of the water in the urine is reabsorbed in the tubules of mammals. Ramsay (1955) has described the reabsorption and salt excretion in the stick insect, *Carausius morosus*. In this animal, the Malpighian tubules produce urine at a rate that it removes the complete water content of the haemolymph in less than a day. However, all the water in the urine is reabsorbed by the rectum.

Osmoregulation in amphibia. In amphibians, water enters osmotically at a slow rate due to the low permeability of skin. The water which enters is excreted as dilute urine. Salt is lost in the urine but is again absorbed from the pond water. The loss of salts is also minimized by active absorption in the kidney tubules and urinary bladder. Passive water reabsorption also occurs which increases the concentration of urine of some waste products that are not absorbed. The tubules of the kidney may also secrete some specific salts as urea. Urine production is under the control of the neurohypophysis, which may altogether stop urine production when the animals stay on land.

Osmoregulation in reptiles and birds. The reptilian skin is covered by scales and loss of water is thus minimized. The water content of tissues of desert reptiles is as high as the mammals. This is maintained by the conservation of water. The renal corpuscles of most reptiles are less poorly vascularized than in aquatic forms. The volume of urine excreted is small in osmoregulate. The capacity to tolerate to such wide range of fluctuations is a special and essential attribute to brackish water animals. When the concentration of the blood lowers, it has been observed in a number of invertebrates that, in tissues (i) there is a slight increase in the water content, (ii) a decrease in the concentration of the ions such as sodium and chloride, and (iii) a decrease in the concentration of free amino-acids and other organic compounds.

Hypo-osmotic regulators

Some animals maintain the salt concentrations of their *body fluids below that of the surrounding environment.* Such animals occur both in sea water as well as inland waters of high salinity. Inland salt waters, such as the Dead Sea, Salt Lake, Utah and the North African Shotts contain a diverse fauna which survives due to their capacity of osmoregulation. Animals which live in inland saline waters can be divided into two groups (Beadle, 1943) (i) Animals found typically in brackish water of moderate salinity, low salinity or even in fresh water, and (ii) animals which exist in high salinities. Crustaceans like *Palaemonetes varians, Cyclops bicuspidata, Hydrobia brondeli* (gastropod) and the fish *Cyprinodon fasciatus* are examples of the first group while the brine shrimp, *Artemia salina* and *Aedes detritus,* the brackish water mosquito belong to the second group.

The composition of the body fluids of animals which follow hypoosmotic regulation varies with the concentration of the medium. In marine and salt water vertebrates, the blood concentration is only slightly higher than that of fresh water forms.

The osmotic pressure of blood is due to sodium and chloride ions whose concentration is higher.

Salt and water balance. The main problems in hyperosmotic medium are (i) *loss of water by exosmosis,* and (ii) *gain of salts by diffusion.* To maintain equilibrium the animals should excrete salts and conserve water.

Salt is removed by secretion into surrounding water. Teleosts and brine shrimp secrete salts into the surrounding medium without any loss of water. Selachians, reptiles, birds, mammals and insects eliminate salts in concentrated solutions losing small amounts of water.

The water lost is replaced by food or by drinking the water in the surroundings. Salt gained is excreted and water is preserved.

The animals which live in hypertonic environment include (i) marine teleost fishes, (ii) marine crabs, (iii) a number of prawns, (iv) larvae of dipteran insects, and (v) the brine shrimps, which live in salt lakes.

Salt secretion in marine teleosts. The teleost fishes living in seawater face the problem of loss of water. Their blood is hypotonic to seawater. In marine teleosts, *Gadus* and *Lophius,* the blood concentration is $-0{\cdot}76$ and $-0{\cdot}77$ respectively, while the seawater is $-1{\cdot}7$ to $2{\cdot}3$. The skin of these fishes, as it is covered over by scales and a coat of mucus, is impermeable and water escapes through gut and gills. If the loss of water thus faced is

Osmoregulation in brackish water animals

Brackish waters are mixohaline ranging between 30% and 0.5% salinity. These waters are restricted to coastal regions, such as *estuaries or salt marshes,* where the salinity often changes with the tides and in larger land-locked seas such as the Baltic, the Caspian and the Aral Sea.

The brackish water forms can be divided into three groups, (i) marine forms which tolerate low salinities, (ii) fresh water animals which can tolerate moderate salinities, and (iii) true brackish water animals which are not found either in sea or fresh waters. The third group can survive in both fresh and salt waters. Marine animals, though tolerate salinities at lower levels can not maintain their normal activities at salinities below 30%. Brackish water forms like *Nereis diversicolor* and *Palaemonetes varians* are not found in seas though they can survive in sea water. On the contsary, brackish water forms cannot survive in fresh water which can be attributed to physiological factors.

Mechanism of osmoregulation. In brackish water forms like *Carcinus* which osmoregulates, the blood concentration corresponds to about 60% sea water when it is living only in 15% sea water. When the concentration of the body fluids is higher than the surrounding medium, *water enters by osmosis and salts are lost by diffusion.* Therefore, to maintain balance, water must be quickly removed from the body and solutes must be replaced. Water is removed through urine and other sources.

Estuarine crabs

Estuarine crabs such as *Carcinus* are euryhaline and home-iosmotic. As the water from rivers enters the sea in the estuaries, the medium becomes dilute and wide fluctuations occur. In contrast to *Maia,* if *Carcinus* is transferred to 75% seawater, there is only a 0.3% increase in weight and it returns to the normal condition within 12 hours. Whereas in *Maia* the internal concentration decreases with the surrounding medium; in *Carcinus* the blood concentration is maintained high, inspite of the dilution of the external medium. In fact *Carcinus* can maintain the blood concentration which is twice the external medium. If the external medium is dilute, the animal increases its rate of output of urine and loses chloride ions steadily. The water enters the body through the gills which are highly permeable and the weight increases only by 0.3% due to high output of urine. Some water is also lost through the alimentary tract also. A hypertonic condition of blood is also maintained by the conservation of salts by ionic regulation. More magnesium and calcium ions are thrown out and potassium ions loss is minimized because of their importance in regulatory activities of the tissues. As the medium is dilute, less salt is present and to maintain a high concentration of blood active absorption of salt takes place through gill surfaces. Thus estuarine crabs are more homo-iosmotic than the marine crabs.

Composition of tissues. In brackish water animals, the composition of the body fluids varies within wide limits through the animals can an equilibrium or until the molal concentrations on the two sides are the same. This movement of water is called as *osmosis*. The *osmotic pressure is the hydrostatic pressure necessary to prevent any movement of water from pure water through a semipermeable membrane into a solution.* As the cell membrane is made up of lipid also and water is insoluble in lipid, it is assumed that water moves through pores or water-filled channels in the membrane. This scheme explains the rapid movements of water through the membrane. The force which moves the water is the osmotic pressure π, defined by *Van't Hoff* expression.

$$\pi = RT\,(Co - Ci)$$

In this the gas constant, R, is equal to 0.08 atm/degree/mole when π is expressed in *atmospheres* of pressure and the concentrations inside (Ci) and outside (Co) are expressed in moles. Osmotic pressure can also be expressed in terms of cm of water or mm of Hg pressure. When a solution is separated from the pure solvent, whose concentration is zero, by a semi-permeable membrane, the osmotic pressure is RTc. T is the absolute temperature.

One of the most convenient methods of the measurement of osmotic pressure is the *depression of the freezing point* measured with very minute quantities of solutions (10^{-5} ml). A one molal solution of an ideal nonelectrolyte in water has a freezing point depression of 1.858°C. Freezing point depressions are used very frequently to determine concentrations and the concentrations are usually expressed in terms of this property which is usually abbreviated to Δ .

Table 6.1 Showing osmotic pressures of the body fluids of animals (expressed as depression of the freezing point) in relation to the habitats in which they live.

Sea = −2·2°C	Fresh water = 0·03°C	Dry land
Invertebrates :	Most fresh water invertebrates	Insects
Blood roughly isosmotic with medium	− 0·4° to − 0·8°	− 0·6° to − 0·8°
	Molluscs − 0·2°	
Vertebrates :		
Teleosts − 0·8° to − 1·1°	Teleosts − 0·5° to − 0·7°	
− 0·8°	Eel → − 0·6° (Migratory)	
	Amphibia − 0·4° to − 0·5°	
Turtles − 0·6°	Reptiles about − 0·5°	− 0·6°
		Birds − 0·6°
Whale − 0·7°	Mammals	− 0·5° to − 0·6°

not regulated, it will lead to the cessation of the life activities in the tissues. The water lost osmotically is recovered by drinking seawater and excreting the salts extra-renally. The salts and water are absorbed quickly by the gut wall and the salts in the seawater raise the salinity of blood, about 3.5% while the tissues of these fishes are adapted for low concentrations of salts. As such, the fishes have to get rid of the extra salt as soon as it enters the body. Kidneys cannot excrete urine which is hypertonic to the blood, thus salt leaves the body through gills, more than that enters the body. The extra salts are excreted from the body by *chloride secretory cells* situated in the gills. This process of secreting salts from dilute blood into seawater is against concentration gradient and requires metabolic energy.

Several anatomical adaptations are found for the production of scanty urine in the marine teleosts. With reduced demand for filteration, the glomeruli are often partially or entirely eliminated. All mesonephroi possess glomeruli during early developmental stages, but the adults are almost aglomerular.

Osmoregulation in marine crabs. Marine crabs, whose blood concentration varies with the fluctuations in seawater are **stenohaline.** Examples are *Maia, Palinurus, Hyas* and *Portunus.* In these animals the renal organs are well developed and occur on the first segment of the second antenna. These are called as the *antennary glands* or *green glands.* A coiled tube starts internally from the coelomic sac and leads to a collecting bladder which opens outside. *Maia* dies in a few hours in 20% seawater, while in 75% seawater it can live for 18 hours. When transferred to the diluted seawater, water enters the animal at such a rate that the weight increases by 2.4% and salt escapes from the body. The blood becomes isotonic with the medium. The reduction of osmotic pressure prevents further entry of water. The kidneys become stimulated by the extra water and start working at a high rate and within three hours, the weight of the crab falls. The urine excreted in large amounts removes chlorides, sulphates, calcium and magnesium. It has been experimentally shown that loss of potassium is the main cause for the death of the crab. Though potassium is excreted in very low concentrations by the kindney, the blood with its reduced amounts of salts cannot sustain the tissues of the animal and death results.

Osmoregulation in migratory fishes. In migratory fishes, like *Anguilla,* both the adaptations to live in freshwater and seawater occur. These fishes which live in freshwater lakes migrate to the sea through rivers and in the sea they may travel up to 1,000 miles. The young larvae of these fishes, the elvers, again migrate to the freshwater lakes. The eels have to regulate their concentration of blood, both in freshwater and in seas. In marine waters the blood concentration is maintained just like in marine fishes, by drinking seawater and secreting the extra salts through the chloride cells and making the urine isotonic with the blood. In freshwater ($\Delta^{-0.08°}$ C) the internal the solute molecules is calculated by the frictional ratio (f/fo), where fo is the frictional coefficient of the anhydrous form of the solute particles . f is derived from the diffusion coefficient $\left[D = \frac{RT}{Nf}\right]$ and fo by the *Einstein-Smoluchowski* equation.

$$fo = \eta \, (162 \pi^2 M\bar{V}) \, N^{1/3}$$

In the above equation, M is the molecular weight of the solute molecule and $\bar{V}$ the partial molar volume.

Diffusion in general is a slow process. But in cells it can be rapid because the molecules of solute have to travel short distances measuring only few Å.

Diffusion through pores

Diffusion can take place through small pores in membranes. In such condition, the influx and efflux mutually interfere. If a solution of the concentration C_1 is separated by a membrane and the solute molecules diffuse through pores at a point 'a' through a point 'b' into a concentration C_2, the number of particles entering at 'a' are proportional to C_1, while the number of particles leaving at 'a' are proportional to C_2 which is a lower concentration. The number of particles entering at 'b' are proportional to C_2 and the number leaving to C_1. Through very narrow pores where particles cannot pass one another and if C_1 and C_2 are both so large that the pore at all times is occupied by several particles, only very few particles penetrate from C_2 to C_1. The probability of each particle entering at 'a' from C_1 to returning to C_1 is $\frac{C_2}{C_1}$ and the probability of moving through the pore is $\frac{C_1 - C_2}{C_1}$. Movement of particles from low concentration C_2 to C_1 can take place very rarely through improbable events.

Exchange diffusion

This is one of the peculiar phenomena which is an exception to the general process of diffusion. In this case, passsage of ions occurs by the mediation of some *carrier material.* The pores in the membrane are impermeable to ions but contain carrier units of ion exchange material which can diffuse freely between the boundaries of the membrane. Ions can pass only in combination with the carriers. If the carrier has high affinity to ions, it becomes saturated. However, each carrier uint can carry one ion in either direction between the two solutions separated by a membrane. If so, the total flux in either direction is equal even when the concentration of the two solutions is different. Pardee (1968) isolated several proteins which acted as carrier molecules.

Pelomyxa has been measured by putting them in capillaries. *Amoeba mira* and *Amoeba lacerata* which are marine and brackish water forms, show good volume regulation within a few hours when transferred to fresh water. When the concentration of the external medium is changed, the freshwater forms, *Amoeba proteus* and *A. lacerata,* fail to return to their normal shape for at least a few hours.

Entrance of water. The surface of protozoans is permeable to water. As a result of the entry of water, the animals swell. This permeability is measured in volume of water entering per unit of the cell membrane for an osmotic gradient in a given period of time. This can be expressed in mol/ml or centimeters of water or osmotic pressure in atmospheres. A pressure difference of 1 atmosphere at 27°C corresponds to a concentration gradient of 1/24,000 mol/cm^3 (Davson, 1959). Some water diffusion constant values for various animals are given below :

Amoeba proteus	... ...	$0{\cdot}42 \times 10^{-10}$
Pelomyxa	... ...	$0{\cdot}275 \times 10^{-10}$
Freshwater peritrichous ciliates	...	$0{\cdot}27 \times 10^{-9}$
Marine peritrichous ciliates	...	$0{\cdot}11 \times 10^{-9}$
Frog eggs	... ...	$0{\cdot}89 \times 10^{-8}$
Arbacia eggs	... ...	$0{\cdot}40 \times 10^{-9}$
Aquatic dipteran larvae	... ...	$0{\cdot}134 \times 10^{-9}$

Water may enter inside the protozoans not only from the surface, but also during feeding. In peritrichous ciliates, inhabiting fresh water, the water taken up by the food vacuoles may be 8 to 20% of the output of the contractile vacuole and in *Paramecium* 30%. In *Paramecium* the activity of the contractile vacuole increases by merely pumping.

Function of the contracticle vacuole. In freshwater inhabitants belonging to the four groups of Protozoa, a contractile vacuole is invariably present, while it may be present or absent in marine and endoparasitic forms. Sporozoans in all cases do not possess a contractile vacuole. In the marine and parasitic protozoans, the contractile vacuole eliminates metabolic water and water that enters along with the food material. Besides this, some water is required to dispose off the excess of salts.

The output of water from the contractile vacuole in freshwater protozoans is more than the marine or endoparasitic forms. The period of time required to eliminate a quantity of water equal to the body volume of the animal for about 15 freshwater forms ranged between 4.1 to 53 minutes. For marine forms the time required was far more than $2\frac{3}{4}$ to $4\frac{3}{4}$ hours.

Experiments conducted by transferring protozoans from sea water to freshwater indicate that contractile vacuoles are absent when placed in seawater and develop in freshwater. *Amphileptus gutta,* a marine ciliate,

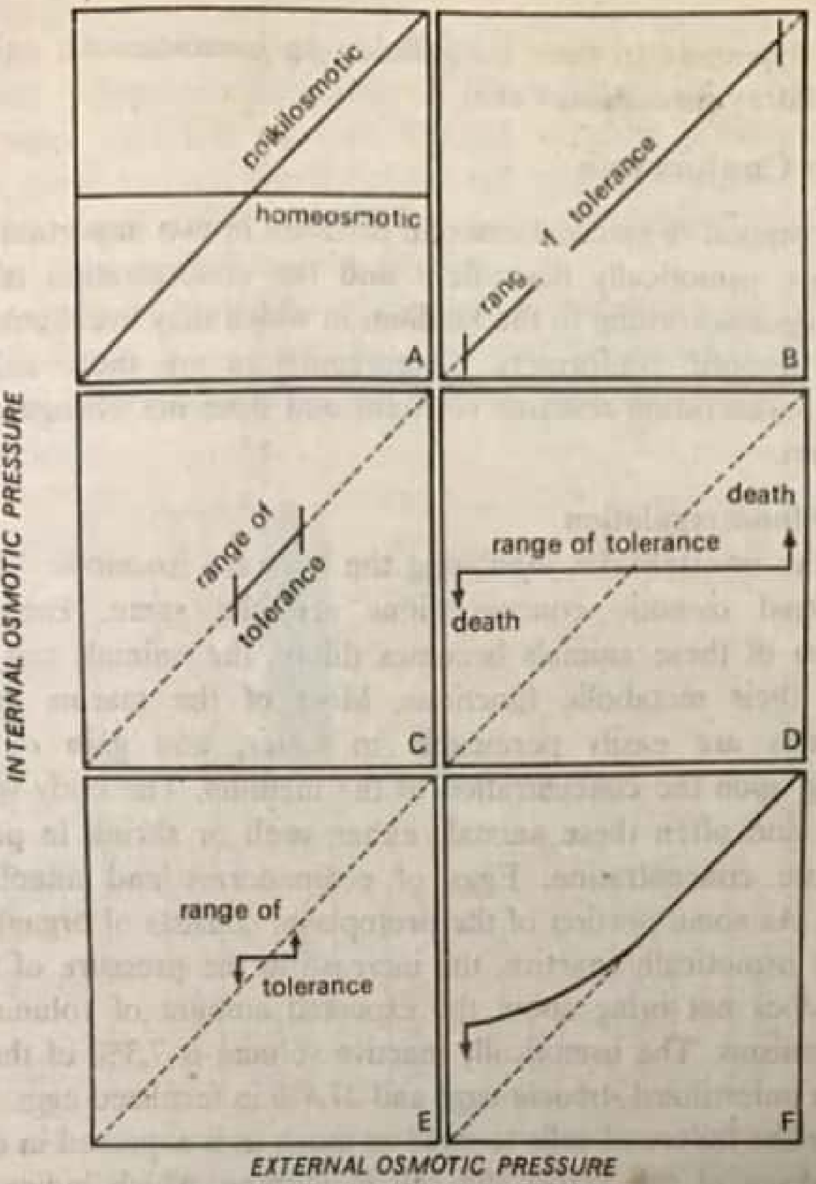

Fig. 6.1. Types of osmoregulation. A—Comparison between homeosmotic and poikilosmotic types. B—Euryhaline poikilosmotic type. C—Stenohaline homeosmotic type. D—Euryhaline homeosmotic type. E—Stenohaline homeosmotic type. F—Hyperosmotic regulation.

Those animals, which survive only within a narrow range of salinities and are bound to constant salt content environment, are called *stenohaline* species. These do not have osmoregulatory mechanisms and cannot maintain a constant internal environment. Aquatic animals whose internal osmotic concentration varies according to the external environment are called *poikilosmotic*. Stenohaline animals are usually poikilosmotic. Many of the euryhaline species are also poikilosmotic but their body cells can withstand the salinity changes in the outside environment and changes in the body fluids. Animals which maintain a constant osmotic concentration of body fluids in a changing external salinity concentration are called "homoiosmotic". Many of the euryhaline species are homoiosmotic, and so are many animals which live in a medium of constant concentration that is

Diffusion

The particles of a solute are under a constant motion in a solvent. The motion can be thermal or *Brownian* movement due to their kinetic energy. In dilute solutions, solute particles constantly collide with solvent molecules and the kinetic energy imparted to the solute particles causes them to undergo random movement through the solution. The particles can travel in any direction. However, if there is a difference in the concentration of the solution at different levels, the solute particles show a *net movement from the region of higher concentration to a region of lower concentration.* Statistical analysis shows the movement of the particles is more from higher concentrations to lower than from lower to higher. *The net movements of the solute due to the presence of a concentration gradient is diffusion.* At equilibrium though the particles of solute move at random, there is no net movement in any direction.

Adolf Fick(1855) proposed the following equation for the flux J of a diffusing solute.

$$J = \frac{ds}{dt} = -DA\frac{dc}{dx}$$

S is the amount of solute in grams of moles passing across an area A, per second. The concentration gradient $\frac{dc}{dx}$ is proportional to the flux. C is the concentration and X the distance. D is the diffusion coefficient which is proportional to flux and is dependent on temperature and the type of solute. D is expressed in the units of $cm^2\ sec^{-1}$. As the net movement is in the opposite direction, i.e., from higher to lower concentration a negative sign is shown.

The diffusion coefficient is dependent on a number of factors like the size and shape of the diffusing molecules, etc. Frictional interactions between solute molecules affect the rate of movement. In a dilute solution diffusion coefficient can be calculated by the formula :

$$D = \frac{RT}{Nf}$$

R is the gas constant, T the absolute temperature, N *Avogadro's* number and f the frictional coefficient which in turn is related to the viscosity η, of the solvent. This can be calculated by *Stocke's* equation :

$$f = 6\pi\eta r^2$$

r being the radius of the diffusing particle. The interrelationships of the diffusion coefficient, frictional coefficient and the viscocity are :

$$D = \frac{RT}{6\,N\pi\eta r^2}$$

Asymmetric molecules decrease the viscocity of a solution as the viscocity is dependent on the friction volume of solute. The sphericalness of

Active Transport

Rosenberg (1954) defined active transport as *the movement of a substance against its eletrochemical gradient.* This process consumes energy. It may be carrier-mediated or without carriers. The energy consumed is equivalent to the product of the electrochemical gradient and the amount of substance moved. Regarding the mechanism, it is believed that on one side of a membrane the carrier combines with an ion, and on the other side, releases it.

Donnan equilibrium

One of the most characteristic features of biological membranes is their *differential permeability.* Some ions like potassium are easily permeable while others like Ca^+ and Mg^+ are less permeable due to their larger size. If two solutions are separated by a membrane impermeable to one of the two ion types, all the ions are not distributed uniformly by diffusion on the two sides of the membrane. Under such conditions, a special type of equilibrium, the *Donnan equilibrium* is achieved.

For example, if potassium chloride (KCl) solution is separated by a membrane from potassium isethionate ($KC_2H_5SO_4$) which is permeable to both potassium and chloride ions and not to isethionate ions, chloride ions move into isethionate by a concentration gradient. But no movement of K^+ ions occurs initially. Movement of chloride ions results in a negative charge in the isethionate solution which attracts potassium ions. The K^+ move against a concentration gradient until an equilibrium is reached. During equilibrium the movement of chloride ions into the isethionate solution is balanced by the movement of K^+ ions back. Thus, the energy produced by the movement of the chloride ions by a concentration gradient is balanced by the energy required to pull same number of potassium ions back into the solution, against a concentration gradient. At equilibrium, the ratios of all diffusible cations on the inside to those on the outside are inversely proportional to the ratio of anions inside to outside. These ratios are equal to *Donnan's* constant.

$$r = \frac{[K^+]^o}{[K^+]i} = \frac{[Cl^-]i}{[Cl^-]^o}$$

Osmoregulation in Animals

Those animals which can withstand a wide range of salt concentrations in the aquatic medium are called *euryhaline* species. This includes *anadromous* animals which migrate from high salt content medium (sea) to low salinity medium (fresh water) and *catadromous* species which migrate from regions of low salinity to regions of high salinity or from sea to fresh water. Most of these two varieties include fishes. The examples are eel and salmon.

Osmoregulation in hypotonic environment (freshwater animals)

In this group are included elasmobranch fishes and several species of crabs whose blood is hypertonic to seawater. They always face the problem of entry of water and loss of salts through outward diffusions. In these conditions, these animals would either swell or lose so much salt that their internal body fluids would not support the proper functioning of the body cells, if protective devices are not developed.

As an important device for adaptation in freshwater, the salt content of the fluids and cells is very low. In *Anodonta*, the blood is isotonic with a 0.1% sodium chloride solution. The reduced salt concentration lowers the osmotic problem.

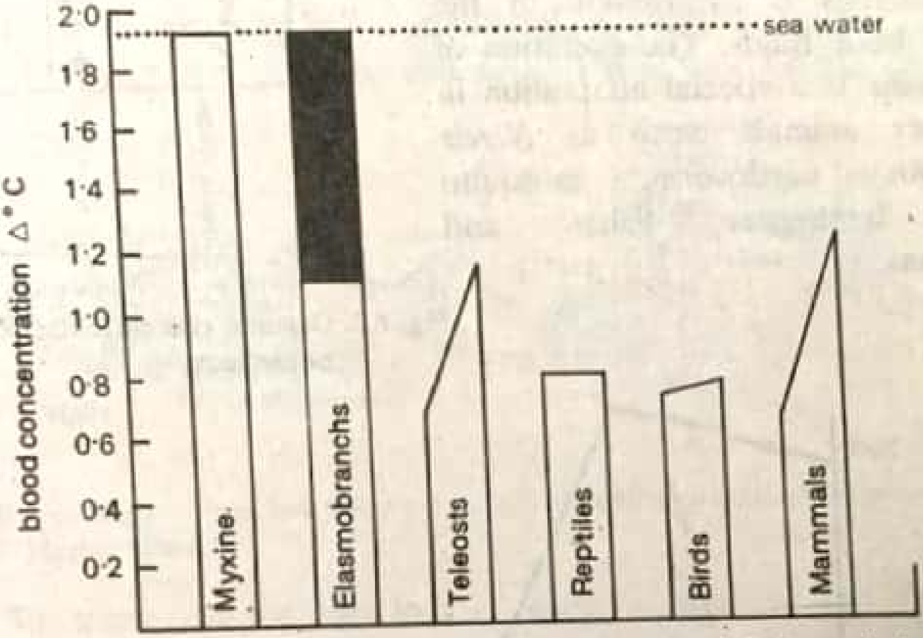

Fig. 6.2. Osmotic concentrations of different marine vertebrates. *Myxine* and elasmobranchs have blood isosmotic with seawater. Black portion denotes the contribution of urea and trimethylamine oxide to the osmotic concentration.

In Figs. 6.2 and 6.3 the salinities of the body fluids of some marine and fresh water species are compared with the salt concentration in the external environment. Another important device is the impermeability of the body wall to water which regulates the entrance of water. In aquatic reptiles, birds, mammals, insect larvae and adult insects, the body wall is absolutely impermeable and their exchange membranes never come in contact with water.

Absorption of salts. The entry of water osmotically creates an internal hydrostatic pressure, which in turn produces increased filtration pressure, resulting in the production of large volumes of urine in freshwater animals. Loss of urine helps in *volume regulation* and thus internal pressure is regulated within limits. As urine consists of salts also, homoiosmotic animals maintain a constant salt concentration in the body fluids. The salt concentration is maintained by active reabsorption of the salts in the urine, hyper or hypotonic to their body fluids. In homoiosmotic animals always osmoregulatory mechanisms exist.

Osmotic Conformers

Animals respond to external osmotic pressure in two important ways. Some animals are osmotically dependent and the concentration of their body fluids changes according to the medium in which they live. Such animals are called as osmotic conformers. Osmoregulators are those animals whose internal concentration remains constant and does not change according to the medium.

Limited volume regulation

Most of the invertebrates inhabiting the seas are isosmotic. Their internal and external osmotic concentrations are the same. Even when the protoplasm of these animals becomes dilute, the animals can survive and carry on their metabolic functions. Most of the marine and parasitic invertebrates are easily permeable to water, and gain or lose water depending upon the concentration of the medium. The body volume is not regulated and often these animals either swell or shrink in proportion to their solute concentration. Eggs of echinoderms and annelids are the examples. As some portion of the protoplasm consists of organic molecules which are osmotically inactive, the increase in the pressure of the external medium does not bring about the expected amount of volume change in these organisms. The osmotically inactive volume is 7.3% of the initial cell volume in unfertilized *Arbacia* eggs and 27.4% in fertilized eggs. The second reason for the failure of cells to swell as much as is expected in dilute media is the leakage of salt across the cell membrane, which indicates that the membrane is not completely semipermeable. Some gregarians from the gut of mealworms swell and shrink according to the tonicity of the medium.

Many multicellular invertebrates also show this poor volume regulation. The body weight of *Golfingia* decreases or increases on transfer to high or low concentrations and reaches the equilibrium in a few hours. The original body volume is restored on return to seawater. About 23% of the body volume is osmotically inactive (Adolf, 1937).

Various degrees of volume regulation are exhibited by molluscs and echinoderms. *Doris* swells repidly in seawater and may remain like that for 24 hours. In *Oncidium,* water permeability is more than salt permeability and therefore little volume regulation. *Aplysia* gains weight in 75% seawater for two to three hours and returns to the normal condition after transfer to pure seawater, showing that it had lost salts during recovery. Echinoderms, such as echinoids, asteroids and ophiuroids due to their hard exoskeleton, cannot swell considerably. The holothurian, *Caudina,* swells in dilute seawater but the increase is much smaller.

by the kidney tubules. Many freshwater animals have highly developed tubules in contrast to marine forms where they are shorter or absent. Freshwater teleosts have nephridia which can reabsorb salts. In freshwater elasmobranchs also similar reabsorption takes place.

Formation of a dilute urine. Because salts are absorbed in the kidney tubules, the urine excreted by these animals is hyposmotic to the animal's body fluids. The excretion of dilute urine is a special adaptation in freshwater animals, such as *Nereis diversicolor*, earthworm, mosquito larvae, freshwater fishes and amphibians.

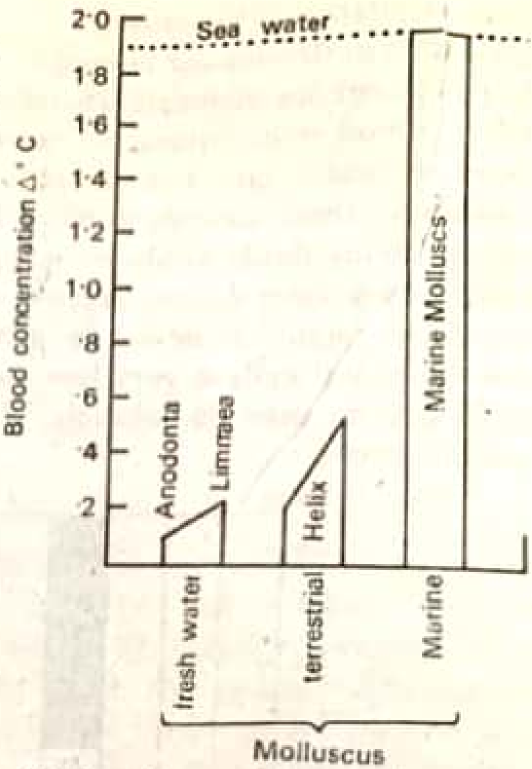

Fig. 6.3. Osmotic concentration of the blood of molluscs.

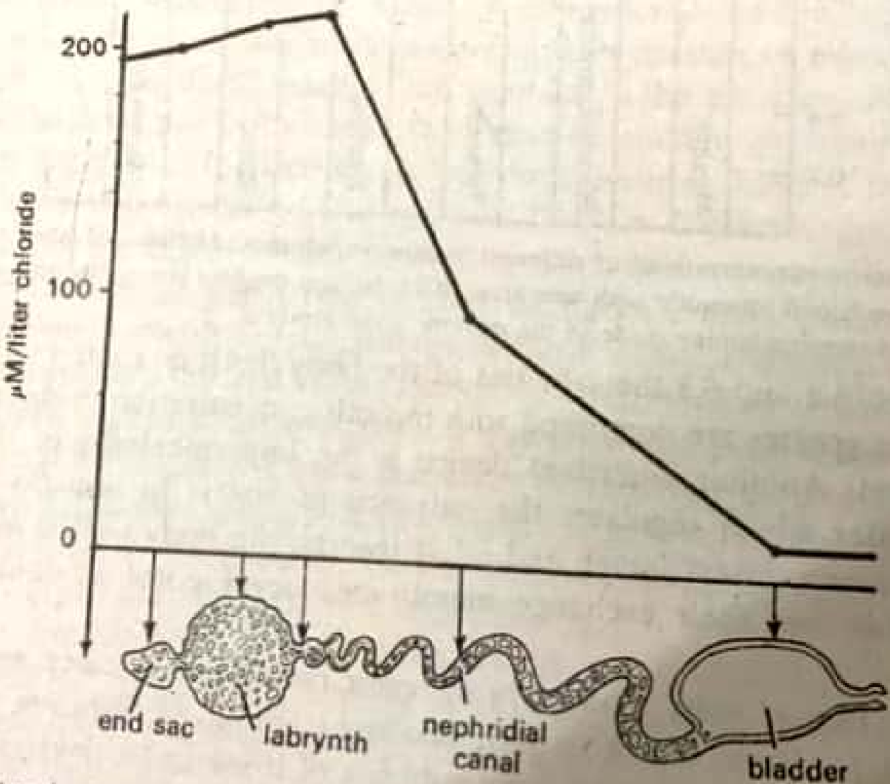

Fig. 6.4. Antenal gland of *Astacus*. Graph represents chloride concentration in different segments. Salt concentration decreases drastically towards the distal portion of tubule and bladder showing reabsorption of salts.

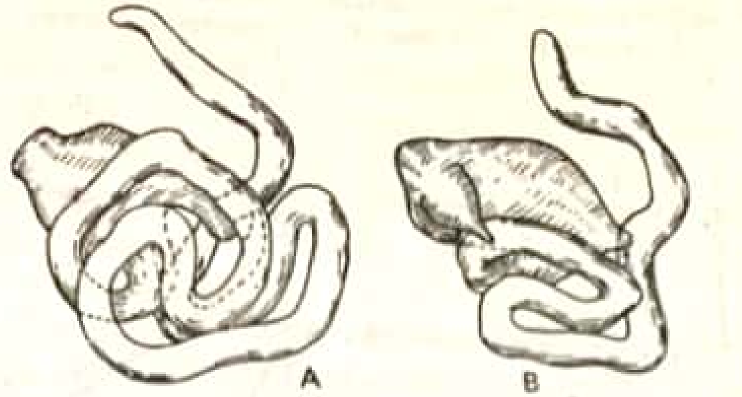

Fig. 6.5 A. Renal organ of *Gammarus locusta* (freshwater), B. The same of *Gammarus pulex*

Absorption of salts by gills. Inspite of the salt conserving devices developed by freshwater animals, still some salt is lost by the excretory

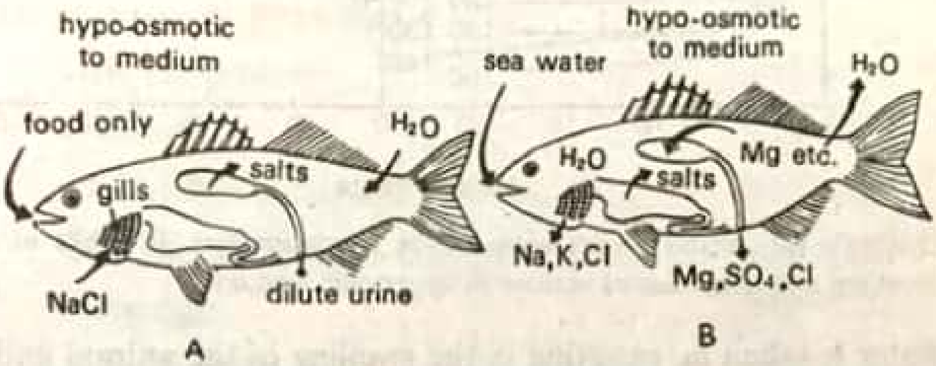

Fig. 6.6. The pathways of ions and water movement in osmoregulation. A. Freshwater fishes. B. Marine fishes.

organs. To make up for the loss of salts, euryhaline and freshwater organisms actively absorb and accumulate salts from dilute solutions. In teleost fishes, amphibia and crustacea, the gills actively take up salt. In *Potamon* and *Eriocheir,* the salt uptake by the gills is about equal to the salt loss by the body surface and through urine (Shaw, 1961). In the larvae of *Chironomus* and *Aedes* the anal papillae absorb salts.

Polkilosmotic and euryhaline animals. In these animals, the osmotic concentration of the body fluids changes with the osmotic concentration of the surrounding seawater. This is effected in two ways. The animals may take up or lose water until the internal medium becomes isotonic with the seawater. Depending on whether the outside medium is hypotonic or hypertonic, the animals may either swell or shrink. The body wall of the sipunculid worms acts as a semipermeable membrane, which is permeable to water but not salts (Fig. 6·7). A similar condition is found in marine molluscs, like *Mytilus, Doris* and *Oncidium.*

Another method is by the excretion of salts. Loss of salts lowers the osmotic concentration of the internal medium to make them isotonic with seawater, and prevents further entry of water. Before the excretion of salts

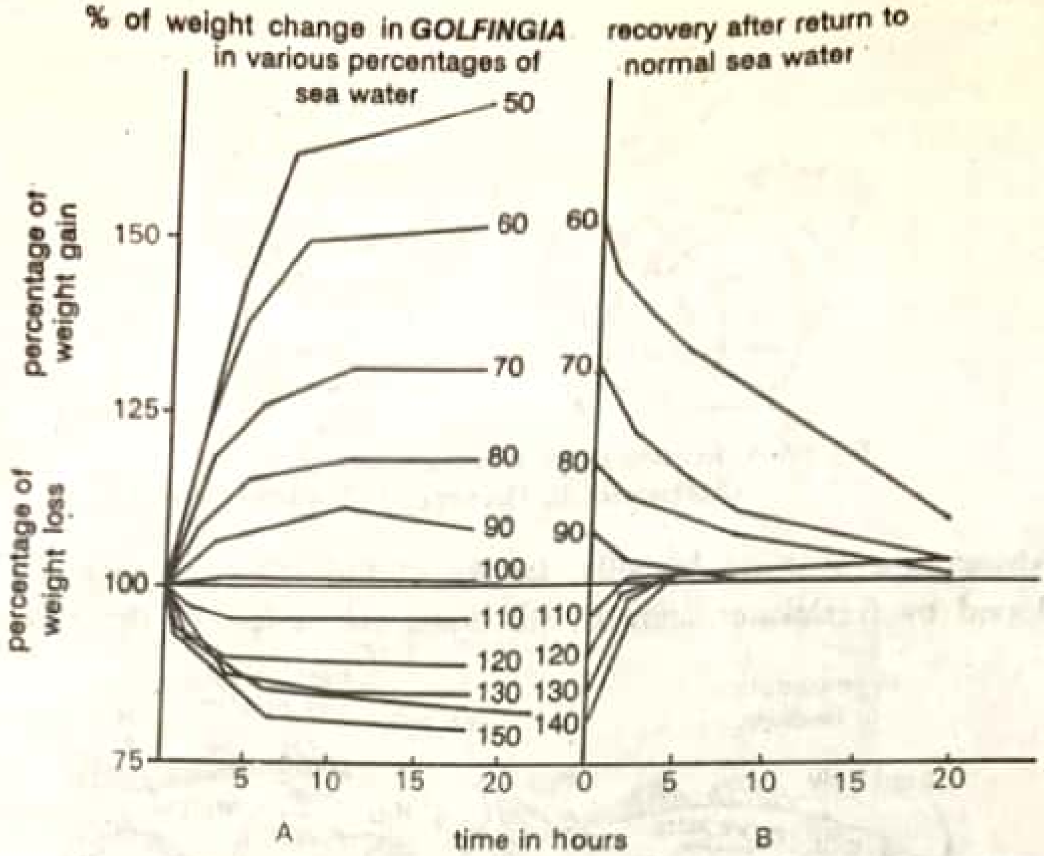

Fig. 6.7. *Golfingia* (sipunculid worm) behaves like an osmometer. It swells in a hypotonic medium and decreases its volume in hypertonic medium.

occurs, water is taken in, resulting in the swelling of the animal and increase in weight. Examples are *Nereis diversicolor* and the marine mollusc, *Aplysia*.

Salt acquisition from food. Food materials mostly contain potassium and sodium ions, both in plant and animal material. Salt conservation in hypotonic medium is aided by the acquistion of salts in the foodstuffs. As the potassium concentration is more than sodium and internal sodium requirements are more than potassium, selective absorption of sodium and excretion of excess potassium occurs. In eels, as the gills cannot absorb salts, food is the main source of salts. Highly impermeable animals mostly adopt this process.

Osmoregulation in Different Animals

Protozoa

Protozoans either freshwater or marine do not face any osmotic problem. Marine and parasitic protozoa are isosmotic with the medium, and eliminate only water which enters along with the food material. Freshwater protozoans are hyperosmotic to their medium and the constractile vacuole eliminates excessive water. Some of the protozoans tolerate extremities in the medium. *Amoeba lacerata* lives freely either in freshwater or seawater. *Paramecium woodruff* can adapt to seawater.

Volume regulation. Osmotic regulation in protozoans is effected by volume change and vacuolar output. The volume of *Amoeba proteus* and showed a rise of 21% in the vacuolar rate in 70% seawater and additional vacuoles were formed in 60% seawater.

The reverse experiment was also found true. Freshwater protozoans when placed in seawater show reduced activity of the vacuoles. *Amoeba verrucosa,* a freshwater form, developed no vacuole in 50% seawater, and on the addition of freshwater the vacuole reappeared. Thus, it is evident that the contractile vacuoles in protozoans have an osmoregulatory function.

Mode of working of contractile vacuole. The mode of operation of the contractile vacuole is not known. How water enters the vacuole from the hypertonic cytoplasm is still a question unanswered. Recent findings show that Na^+ and K^+ ions are involved in ion and water regulation in protozoans. Protozoans in fresh water live under hypotonic conditions and the main problem faced is how to prevent the inflow of water and if water enters, how to get rid of it ? They cannot develop impermeable membranes because of the need for exchange of gases and nutrients. Alternatively, they develop active transport processes to accumulate ions in cells.

Potassium, for instance, is accumulated in the freshwater forms like *Acanthameba, Chaos, Spirostromum, Paramecium,* and *Tetrahymena. Chaos* and *Tetrahymena* are known to transport sodium actively out of the cell via contractile vacuole. The sodium accumulation within the cell is sometimes more than 8 times greater than in the cytoplasm. This suggests that Na^+ enters actively into the contractile vacuole which raises the osmotic concentration above the cytoplasm. Due to this, water enters the vacuole but how it is discharged outside of the cell is not yet known. The aggregation of a number of mitochondria around the vacuole seems to suggest that the energy of electron transport may be useful in this process.

Studies of the potential difference with microelectrodes in *Amoeba* show a positive charge of 20 mv with respect to cytoplasm which suggests that this may also play an important role in the functioning of the vacuole.

Freshwater crabs

Freshwater crabs live in a medium which is hypotonic to blood, i.e. the salinity of the blood is more than that of the seawater. The adaptations listed for animals living in hypotonic medium occur in these animals. They are : (i) more water is excreted in the form of dilute urine. However, the urine, though hypotonic to blood, is hypertonic to freshwater. Thus some of the salts are lost; (ii) the loss of salts is made good by kidney or antennary gland, where reabsorption of salts takes place; (iii) an ionic regulation takes place. When the blood is concentrated, more potassium and calcium and less magnesium are present than in the medium suggesting a regulation of mineral composition of blood; (iv) salts are also procured from food material; and (v) absorption of salts takes place by gills also. Through all these devices the salt concentration of the internal medium is maintained at a proper level.

terrestrial forms which may be even semi-solid. The cloaca may reabsorb water. In land reptiles loss of water is more by way of lungs and desert reptiles get most of their water from food. Lizards and snakes often drink water to compensate its loss. Marine reptiles have special glands in the head with openings in lachrymal of nasal ducts. These glands secrete salts and thus the excess salts of the body are removed.

In birds, the cloacal urine is a viscous paste of uric acid crystals. Water absorption may take place in the cloaca, lower intestine and kidney.

Osmoregulation in mammals. Mammals live in varied environmental conditions. Regulation of water content permits them to live in moist or dry air in fresh or salt water and over a wide range of temperatures. The kidney tubules produce a blood hyperosmotic urine. The osmoregulation in mammals is closely associated with the temperature control of the body and is regulated by endocrine and nervous systems.

In man, the daily loss of water by kidneys faeces, evaporation, sweat and lungs amounts to one to nine litres, depending on temperature, exercise and other factors. This loss is compensated by water drunk, water in food and metabolic water. Urine production in man is dependent on the temperature and body water load. The permeability of the skin to water is extremely low, which is very important in aquatic mammals. Many mammals, however, use water evaporation by sweat glands as a means of cooling. Reabsorption of water also takes place in the kidney tubules. Those mammals which produce the most concentrated urine have the longest loops of Henle as absorption takes place in this part. In man 1,100 litres of plasma circulated per day in the glomeruli yields 180 litres of filtrate, out of which 178·5 litres are absorbed leaving 1·5 litres per day of urine.

SASTRI

Important Questions

1. How has the development of osmoregulatory mechanisms affected animal evolution ?
2. How do marine and freshwater fish maintain osmotic homeostasis ?
3. Discuss the osmoregulation in migratory fishes.
4. What is osmoregulation ? Write an account of the regulatory processes in different groups of animals.
5. What are the adaptations in terrestrial animals for water conservation ?
6. Differentiate between : (i) Ewyhaline and stenohaline animals (ii) Osmotic conformers and osmoregulators (iii) Volume regulators and volume conformers (iv) Poikilosmotic and homeosmotic animals.
7. Explain why marine fish can drink sea water and survive but humans cannot.