INTRODUCTION AND DEFINITIONS

The building unit of living organisms, the cell, can as a very crude first approximation be described from the point of view of osmotic conditions as a solution contained within a membrane. Surrounding the cells in multicellular animals we have similar solutions contained within the cellular integument and constituting the “internal environment” of the cells. With insoluble constituents of the organism we are not here concerned. The “solvent” is always water which is present in very variable proportion, from over 99 to less than 50% of the total mass of solution.

Going a step further we can distinguish between dissolved particles which are of the same order of size as the water molecules and move at similar rates and the much larger colloidal particles, in the organisms almost always of a protein nature, which are in comparison practically immobile. The small particles + the amount of water in which they are free to move is often called the “continuous phase” and distinguished from the “disperse phase” constituted by the colloidal particles and the “bound” water. The question of bound water is rather controversial, but in most cases the quantity seems to be small, so that we can consider practically all the water present as solvent water.

In the present monograph interest centres upon the relations between the concentrations of solutions, inside and outside cells and organisms. The “total concentration” is given by the number of particles present in a litre of solution or in a kilogram of water. It is expressed in moles, and 1 mole of any substance, the number of grams corresponding to the molecular weight, means $6 \times 10^{23}$ ultimate particles. A solution containing 1 mole of particles, irrespective of their chemical nature, per litre is called a “molar solution”, and a solution containing 1 mole per kg. water is called a “molal solution”. In fairly dilute solutions like the water in nature and the body fluids of many animals there is no significant difference between concentrations expressed by molarity or molality and we shall not treat it seriously; but in more concen-
treated solutions, like the blood of higher animals and the content of most cells, the distinction becomes important, mainly because the colloidal particles make up a large fraction of the mass, but, on account of their relatively enormous size, only a small fraction of the number of dissolved particles. In such cases we express the concentration of substances in true solution by their molality, assuming as the solvent the quantity of water which is expressed by the difference between fresh weight and weight of dry substance. No account is taken of the possible presence of bound water.

Very important physical properties of solutions are quantitatively dependent upon their total (molal) concentration. The one in which we are mainly interested is the “osmotic pressure” which is usually defined by means of the properties of a membrane called “semi-permeable”, which will allow water molecules to pass through, but will hold back all dissolved substances. If a solution is enclosed in rigid, semipermeable walls and surrounded by pure water the inside pressure will attain the value of 22.4 atmospheres per mole held in solution by 1 kg. of water. At this pressure equilibrium is established between the quantity of water “attracted” by the dissolved molecules inside and the water filtering out by reason of the “hydrostatic pressure” inside. When the outside fluid is not pure water, but a solution, the pressure developed will be proportional to the concentration difference, and when the concentrations are made equal there will be no pressure.

It is deeply significant that the conception of osmotic pressure was introduced by a physiologist studying plants (Pfeffer, 1877), who also succeeded in constructing osmometers with semi-permeable membranes and establishing experimentally the relation between concentration and osmotic pressure. The plant cells are natural osmometers having a rigid wall of cellulose supporting a protoplast which is practically semipermeable and contains in many cases one large vacuole. The concentration of the cell sap sets up by attraction of water a hydrostatic pressure generally of many atmospheres and responsible for the turgor of plant tissues. When such cells are tested by means of solutions of increasing strength, a concentration can be found which will reduce the inside pressure to 0 and cause the protoplast just visibly to recede from the cellu-
lose wall, and this concentration is a measure of the concentration within the cell. A further increase in outside concentration brings about a definite shrinkage of the protoplast.

In animal cells there is, with few exceptions, no mechanically supporting structures, and only a very slight hydrostatic pressure, amounting to some centimetres of water, can be borne by the protoplasmic membrane. When such cells are at all permeable to water (and impermeable to solutes) it is a necessary consequence that the concentration must be the same inside as outside, and when the outside concentration is lowered water must flow in and dilute the content, until equilibrium is restored by swelling. Shrinkage must take place for the same reasons when the outside concentration is raised above the inside. A number of exceptions to this general rule will be discussed in the following.

To measure osmotic pressures of solutions, whether artificial or obtained from organisms, directly by means of semipermeable membranes in artificial osmometers is a very difficult proposition. In some cases determinations can be made by swelling or shrinkage experiments on the animal cells themselves, but in most cases indirect methods have to be applied. These take advantage of one or other of two facts.

The water in a solution freezes at a lower temperature than pure water, and the freezing-point depression $\Delta$ is at fairly low concentrations directly proportional to the total concentration, a molal solution freezing at $-1.86^\circ C$. Determinations of freezing-point depressions are theoretically very simple, but where biological fluids are concerned there are several difficulties, referred to in the Appendix on methods.

The water in a solution has a lower vapour tension than pure water. The difference in vapour tension is very small and a direct measure is generally not feasible, but comparisons can be made with known solutions according to two different principles. One utilizes the fact that when two solutions of different concentration are placed side by side the more dilute will evaporate at a more rapid rate and therefore have a lower temperature, which difference can be measured by a suitable thermocouple (Appendix, p. 211). Measurements according to this method can be carried out in a
short time. In the other method changes in volume are used, since water will be transferred as vapour from a more dilute to a more concentrated solution. This transfer is so slow that ordinarily measurements will require 24 hr. or more.

While the freezing-point determinations yield absolute values, the vapour-pressure determinations are always carried out as comparisons with known solutions, and it has become customary in biology to use solutions of NaCl for the purpose and to express concentrations by the osmotically equivalent NaCl concentration.

NaCl as a reference substance has certain drawbacks. As an electrolyte it is in dilute solutions completely dissociated into the ions Na⁺ and Cl⁻, and each molecule should act as two separate particles, but the observed osmotic activity is somewhat lower and the reduction depends to a certain extent upon the concentration, being larger in more concentrated solutions. This will affect the recalculation of freezing-point depressions into molar concentrations. I have on the whole disregarded the concentration factor and used the relation 0·293 mole Cl⁻ (or Na⁺) = 1·00° freezing-point depression as giving a sufficient accuracy for the problems here under discussion.

By determinations of total concentrations we can find out whether the internal medium of an organism is in osmotic equilibrium or not with the external medium and with the continuous phase within cells, and, when differences are found, we can draw certain inferences regarding the movement of water from one solution to another, but in a large number of cases we are also interested in the distribution of single dissolved substances, and the concentrations of these must be expressed also by moles when different neutral or charged particles (ions) are to be compared. As the mole unit is inconveniently large for our purposes I use throughout as unit of concentration the millimole (designated mM.). A 1 mM. solution of NaCl means a solution containing 1 mM. of Na⁺ + 1 mM. of Cl⁻ per litre and a 1 mM. solution of Na₂SO₄ contains 2 mM. of Na⁺ and 1 mM. of SO₄²⁻ per litre. The atomic weights by which the weight units used in many papers are converted into mM. units are Na 23·0 mg., K 39·1 mg., Ca 40·1 mg., Mg 24·3 mg., Cl 35·45 mg., S 32·1 mg. and P 31·0 mg. When no
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misunderstanding is possible quantities of substances are also
given in molar units, and it has been found convenient generally
to use the micromole (designated \( \mu \text{M} \)) as the unit. \( 1 \ \mu \text{M} \) of sodium
means 23 micrograms (\( \gamma \)).

We have to deal in the organism only with neutral solutions in
which the kations and anions balance each other practically com-
pletely. It has been found convenient, though undeniably incon-
sistent, to denote the total concentrations of ions by the sum of
either kations or anions, although of course the real concentration
is double this figure and the osmotically effective concentration
somewhat less than double.

Osmotic flow of water. As referred to above any difference in total
concentration across a semipermeable membrane will cause a flow
of water through the membrane, and it is possible and desirable in
several cases to express quantitatively the permeability of such
membranes. This can be done by measuring the rate at which water
will penetrate under pressure, whether osmotic or hydrostatic. The
rate is evidently proportional to the pressure difference between
the two sides of the membrane and the area exposed, and inversely
proportional to its thickness. In biology we have to disregard the
term “thickness” which can in most cases not even be defined, and
it has become customary for artificial membranes used in ultra-
filtration experiments to express permeability by the minute num-
ber which, being inversely proportional to the rate of water pene-
tration, is the time necessary for 1 cubic centimetre (cm.\(^3\)) to pass
through 1 square centimetre (cm.\(^2\)) of membrane by a pressure
difference of 1 atm. This time is for filters which will retain bac-
teria less than 1 min. and for collodion membranes which will filter
off practically all colloids of the order of 100 min. For the animal
membranes with which we have to deal the time varies from a few
days (of 1440 min.) to a few years.

Absolutely semipermeable membranes in the sense defined
above probably do not exist at all. Organic membranes which are
found impermeable to water are somewhat permeable to gases like
\( \text{O}_2 \) and \( \text{CO}_2 \), and it is the rule for membranes having “minute
numbers” of a few days or weeks to be slightly permeable at least
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for certain ions. The permeability to organic substances is in many cases not related at all to the permeability to water. The water permeability of an organic membrane is not an invariable quantity. It usually changes with time, and it is often highly dependent upon the nature of the solutions bathing its surfaces. Many examples, illustrating this point, will be given in the following.

Diffusion of solutes. When two solutions containing the same substance in different concentrations are separated by a membrane permeable to that substance, diffusion will take place from the solution having the higher concentration towards the solution with the lower and go on until the concentrations shall have become equal.* Several substances may diffuse simultaneously and even in opposite directions, and the exchange by diffusion is also largely independent of osmotic differences in total concentration, although of course diffusion against a flow of water is retarded. For electro-neutral substances like urea or glucose the exchange by diffusion is theoretically a simple affair. For charged particles conditions are more complicated. Suppose we have on the two sides of a membrane permeable to cations, but not to anions, equal concentrations of NaCl and KCl respectively, an exchange of Na with K will take place, but the rate will be governed by that ion (Na) which diffuses most slowly, because a more rapid transfer of K ions would set up electrostatic forces sufficient to keep back the fast-moving ions until they could be exchanged in equal amounts with Na ions. Suppose again that we had KCl and CaCl₂ respectively, and that the membrane was permeable to K⁺ but not to Ca²⁺, then no diffusion could take place. If, however, hydrogen ions or ammonium ions were produced on the side of the Ca²⁺, these would pass out in exchange with K⁺. Because of the complications briefly referred to, because of difficulties in defining and reproducing the properties of living membranes and also for technical reasons, measurements of diffusion rates for ions through living membranes

* This does not hold absolutely when other substances are present which do not pass the membrane. In such a case, as for instance between a solution containing protein and an “ultrafiltrate” from the same, there will be slight differences in concentration of single ions and we speak of a “Donnan” equilibrium between the two solutions. We can, in almost all cases, afford to disregard differences caused by Donnan equilibria.
are completely lacking, and the most that can be done is to arrange ions in the order of their rate of penetration through a definite membrane.

In recent years "heavy water", D₂O, has been utilized for the study of permeability. D₂O in ordinary water on one side of a membrane will diffuse like any other dissolved substance, and it is comparatively easy to measure the rate of diffusion. It is necessary to point out, however, that it is not possible to figure out the rate of osmotic water transport from diffusion experiments with D₂O. According to Jacobs (1935, p. 79) the two processes are of a different nature. When, however, a membrane is found to be impermeable to D₂O it is legitimate to conclude that it is also water impermeable, and very large differences in the diffusion rates for D₂O will indicate at least a difference, going in the same direction, in the rate of osmosis.

Active transport of substances across membranes. In living organisms concentration differences can be maintained in spite of the permeability of membranes, such as is the case in all water-permeable organisms living in fresh water and keeping up at an approximately constant level a much higher total concentration than that of the surrounding water. This can be done only by the steady expenditure of energy in special mechanisms adapted for the purpose. In such a case there is no equilibrium between the internal and external medium, but we use the word "steady state" to characterize the situation.

The main object of this book is to describe the osmotic and ionic steady states encountered in aquatic animals, to locate the mechanisms by which they are maintained, and to describe, as far as that is found possible, their mode of working. Such description is in the present state of affairs very incomplete.

With regard to cells I have stated above the general rule that they are in osmotic equilibrium with their surroundings. The concentrations of single ions in the cell water are, however, in almost all cases widely divergent from the outside concentrations. We have in the body fluids of most animals a great excess of Na⁺ and Cl⁻ over all other ions. K⁺ makes up a few per cent, at most, of the
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total kations and HPO$_4^{2-}$ an infinitesimal fraction of the anions, but 
the state inside the cells is often completely reversed, K$^+$ and 
HPO$_4^{2-}$ being in excess, and generally greatly, of all other ions 
present. We have, therefore, an ionic steady state along with os-
motic equilibrium.

The body fluids which make up the environment of most of the 
cells in multicellular organisms are themselves separated from the 
external medium by membranes which are usually cellular, and the 
cells of which are bathed on the inside by body fluid, on the out-
side by the external medium. When there is osmotic equilibrium 
between the external and internal medium, and when this latter 
follows passively concentration changes in the external, we design-
nate the animal in question as “poikilosmotic”. A very large num-
ber of marine invertebrates are not only poikilosmotic, but the ionic 
composition of their body fluids shows only insignificant 
deviations from that of the sea water surrounding them. Certain 
other marine animals are poikilosmotic, but the ionic composition 
of their body fluids shows definite deviations from that of the sur-
rounding sea water.

When animals maintain a total concentration of their body fluids 
different from that of the surrounding water they can be termed 
“homoiosmotic”, when it is remembered that there are all possible 
transitions between complete independence of the internal con-
centrations from that of the external and the poikilosmotic state. 
Fresh-water animals are, in this broader sense, without exception 
homoiosmotic.

The term “stenohaline” is used for animals which can live only 
within a limited range of outside concentrations, while “eury-
haline” forms can live over a wide range—either by toleration, 
when they are poikilosmotic, or by regulation of the internal con-
centration.

It has been attempted to arrange the material presented on the 
following pages according to the physiological viewpoints sketched 
above, but the task proved too difficult. The solutions of osmotic 
problems actually encountered in nature were too diverse to fit into 
any “rational” scheme, and the simple plan had to be adopted of 
presenting results within the frame of the zoological system. This
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arrangement at least brings out one fact which it is important to keep in mind, viz. that our knowledge is extremely meagre and fragmentary and that it may well be possible to find animals better suited as objects for the solution of special problems than the few species hitherto experimented on.

The material is accordingly divided into chapters corresponding to the major systematic groups of the animal kingdom. A special chapter is devoted to the osmotic conditions in eggs and embryos.

In a final chapter the whole of the material is reviewed, and certain general conclusions presented, with suggestions concerning the most useful lines of future work.

In an Appendix a selection of methods used and proposed in the study of osmotic and ionic regulation are briefly described and commented on.
PROTOZOA

In the phylum Protozoa we are confronted with the major problems of osmotic regulation, but while we find that the tiny organisms have solved these problems for themselves, our task of getting an insight into the solutions is made exceedingly difficult by the very minuteness of the mechanisms concerned.

Among the Protozoa certain forms are morphologically simple and can be considered as primitive, but others possess highly complicated structures and must have been evolved from a long line of ancestors showing increasing complexity. In all the larger groups we have both marine and fresh-water forms, and genera adapted to the peculiar conditions of a parasitic existence are also quite common. The minute size involves an extremely large surface per unit volume. To illustrate this very important point let us calculate the surface per cm.\(^3\) of cubes of 0.001, 1 and 1000 cm. Such cubes have volumes of 10\(^{-9}\), 1 and 10\(^9\) cm.\(^3\), and surfaces of 6 \times 10^{-6}, 6 and 6 \times 10^6 cm.\(^2\). The surface per cm.\(^3\) is therefore respectively 6000, 6 and 0.006 cm.\(^2\), and we have generally that when linear dimensions are reduced or increased the surface areas per unit volume will change in the inverse ratio.

In the Metazoa we distinguish between the internal environment immediately in contact with the cells and the external in contact only with the body surface. In this sense there is no internal medium for the Protozoa, the cells being directly exposed to any variation in the external medium. We are concerned here primarily with variations in the molar and ionic composition of the medium, but with other variations only in so far as they result in modified reactions on the part of the organisms to the ionic or osmotic environment. Certain organisms, both among Protozoa and among Metazoa, are extremely sensitive to changes in the environment, while others will stand large variations, and in this latter case the power of endurance may lie either in a power of toleration or in a power of resistance or in both.

It is important to note that the plasma membrane of Protozoa