Donnan Equilibria in Biological Processes.

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THE LIVING CELL AS AN OSMOTIC SYSTEM AND ITS PERMEABILITY TO WATER.[36]

IF the living cell behaves as an osmotic system, we can apply thermodynamics, and the values of the constants like molecular weight obtained by using the cells as osmometers, should agree with those found by means of non-living systems.

A typical mature plant cell may be considered as consisting of a solution of various electrolytes and non-electrolytes (the cell sap) enclosed in a semipermeable membrane, the extremely thin protoplasmic layer (protoplasm). The latter forms a closed sac lying against the semi-rigid cellulose wall (the cell wall) which in most plants is permeable both to water and solute. The pressure exerted by the sap keeps the cell wall under a certain tension to which is due the normal state of rigidity (turgor) of the cell. When a plant cell is placed in pure water or in a solution hypotonic with respect to its contents, entry of water due to end-osmosis is generally prevented by the rigidity of the wall. When on the other hand, a cell is placed in hypertonic solution, exosmosis occurs, the sap diminishes in volume and the encircling protoplasmic layer retreats from the cell wall, which loses its tension. By placing cells of the same kind in solutions of graded concentration it is possible to find for each solution that particular concentration in which plasmolysis is barely perceptible.

H. de Vries[37, 38] using the leaf cells of *Tradescantia discolor* as osmometers determined the molecular weights of raffinose and sucrose. Similarly, the cells of *Spirogyra* were used as osmometers by Overton.

Probably no other kind of animal cell has been as extensively employed for investigation of osmotic phenomena as the red blood cell. These isolated cells are easily obtained at any time and in any desired quantity, they have relatively a simple structure, they can be kept in usable condition for a longer period of time than any other kind of cell, and unlike many other isolated cells erythrocytes show no seasonal variation. Its osmotic properties are perhaps more influenced by slight environmental changes than is the case with any other kind of cell. In common with other cells the erythrocyte is freely permeable to a great variety of organic compounds whereas it is impermeable to salts as such. However, unlike most other cells, the erythrocyte is permeable to many anions, so that the exchange of anions may take place between the cell and its surrounding medium. For example, it is well known that HCO_3^- when its amount increases in the blood corpuscle, is exchanged for Cl^- from the plasma. Hamburger[39] first established the fact that the relative osmotic pressure of solutions may be determined by using the erythrocyte as osmometer. Equally convincing experiments were performed by Kœppe,[40] Hedin,[41] and others.

As in the case of plant cells it is possible with a muscle to determine the strength of a solution in which the muscle neither gains nor loses water (a solution which is isotonic with the muscle). Such experiments show that both plant and animal cells obey the laws of osmosis (based on thermodynamics) so long as they remain alive and uninjured, i.e., as long as the semipermeability of their surfaces is preserved.

Similar experiments have been performed with leucocytes and spermatozoa,[42-44] muscles such as the gastrocnemius or sartorius of frogs,[45] with certain plant cells of regular geometrical form like the parenchymatous cells of the stem of *Tradescantia elongata*,[46]

[36] Lucké and McCutcheon, *Physiological Reviews*, 1932, **12**, 68.

[37] H. de Vries, *Zeit. Physik. Chem.*, 1888, **2**, 415.

[38] H. de Vries, *Bot. Zeit.*, 1888, **46**, 394.

[39] Hamburger, *Arch. F. Physiol.*, 1886, 476.

[40] Kœppe, *Zeit. Physik. Chem.*, 1895, **18**, 261.

[41] Hedin, *ibid.*, 1895, **17**, 164.

[42] Hamburger, *Archiv. F. Physiol.*, 1898, 317.

[43] H. Kœppe, *ibid.*, 1895, 154.

[44] H. Kœppe, *ibid.*, 1899, 504.

[45] R. Beutner, *Biochem. Zeit.*, 1913, **48**, 217.

[46] K. Höfler, *Ber. deutsch. Bot. Gesellsch.*, 1917, **35**, 706.

with yeast cells,[47] and also with egg cells of Echinoderms, *Arabacia punctalata*.[48]

Cramer[49] has formulated the problem of permeability of water as follows : "Is the water content of the living cell dependent entirely on osmotic forces or is it dependent also on the inhibition of the lyophilic colloids of the cell content ?" In the former case, the water would be entirely free, in the latter a fraction of water would be bound and incapable of taking part in osmotic exchange. Gortner,[50] however, points out that there is 'an insensible gradation from water which is an integral part of protoplasmic structure to that which is simply the medium in which the protoplasm is suspended '.

It is well known that electrolytes play a prominent rôle in the properties of cells. The effect of low concentrations of electrolytes on permeability to water has been investigated by the use of unfertilised *Arabacia* egg.[51] These experiments clearly showed that chlorides of univalent cations tend to increase permeability to water, while those of bivalent cations tend to reduce permeability, to its value in sea-water. It is reasonable to infer that the low permeability values obtained in sea-water are dependent on the presence of Ca and Mg.

Effect of activation (fertilisation) of echinoderm eggs on the permeability to water has been investigated, and of the many chemical and physical changes that occur in the egg cell on fertilisation, its increased permeability to water-soluble and diffusible substances is regarded by Lillie[52] as of fundamental importance. Evidences of such permeability change, quoted by Lillie, are increased electrical conductivity, the readier entrance of substances like alkali and certain dyes, the loss of certain substances (*e.g.*, pigment), from the fertilised egg, and the fact that pure isotonic solutions of sodium salts, which are known to increase permeability and to serve as activating agents, do neither in the presence of calcium or magnesium salts or anæsthetics.

In this connection the observation of Loeb[53] is recalled that parthenogenetic agents are in general cytolytic ones. This is true of the pure salt solutions and acids (*e.g.*, butyric) ordinarily used to activate the egg artificially, as well as agents less often used, such as electric currents, heat and mechanical stimulation. Lillie sees a close connection between the activating effects of these agents and their stimulation of irritable tissues. In the case of both activation and stimulation he regards the first step as the increase in permeability of the cell surface, as the result of which occurs a change in electrical polarisation ; electrical changes, he regards, as the critical phenomena which brings about either activation or stimulation.

Lillie also concludes that fertilised eggs are several times more permeable to water, than are unfertilised ones. The increase in permeability is not instantaneous. It begins probably 2 to 4 minutes after insemination and is in greater part completed during the next 10 minutes.

The permeability of the unfertilised egg of the seaurchin, *Arabacia*, is about 0·1, in the fertilised state it increases two to four times, while the permeability for the human red corpuscles is of the order of magnitude 3·0. In membranes of living systems an extreme range of permeability is found from practically complete impermeability to free permeability. Thus, under certain conditions, the membrane of the egg of *Fundulus* is practically impermeable to water ; the other extreme is found in the cellulose wall of many plant cells which permit free diffusion of water.

DONNAN EQUILIBRIUM IN BLOOD-CORPUSCLES.[19,20]

E. Warburg[15] was the first to apply the theory of Donnan Equilibria to red blood-corpuscles, and considerable progress has been made during the last few years.[16,17] It is well known that the red corpuscles do not possess any nucleus, and as a result the metabolic processes are either completely absent or play an unimportant part. Further these cells are impermeable by micellæ and simple cations like Na^+, K^+ but are permeable to anions like HCO_3^-, Cl^-. Thus all the necessary conditions for Donnan

[47] K. T. Wieringa, *Protoplasma*, 1930, **8**, 522.

[48] McCutcheon and Lucké, *Journ. Gen. Physiol.*, 1931, **14**, 393.

[49] W. Cramer, *Trans. Farad. Soc.*, 1930, **26**, 686.

[50] R. A. Gortner, *ibid.*, 1930, **26**, 678.

[51] McCutcheon and Lucké, *Journ. Gen. Physiol.*, 1928, **12**, 129 and 571.

[52] R. S. Lillie, *Amer. Journ. Physiol.*, 1916, **40**, 249.

[53] J. Loeb, *Science*, 1913, **37**, 427.

equilibrium are apparently fulfilled, and this equilibrium condition between the liquid within the cell and the serum outside it may be represented diagrammatically as follows :

Red corpuscle.　　　　　　　Serum.

$$
\begin{array}{ll}
\text{Hb} & \text{P} \\[4pt]
\left.\begin{array}{l}\text{Hb}^- \\ \text{Cl}^- \\ \text{HCO}_3^-\end{array}\right\} \text{B}^+ \left\{\begin{array}{l}a \\ b \\ c\end{array}\right| & \left.\begin{array}{l}x \\ y \\ z\end{array}\right\} \text{B}^+ \left\{\begin{array}{l}\text{P}^- \\ \text{Cl}^- \\ \text{HCO}_3^-\end{array}\right. \\[12pt]
\hspace{1.5cm}(1) & \hspace{1cm}(2)
\end{array}
$$

where B^+ = sum of the cations Na^+ and K^+.
　　Hb = hæmoglobin.
　　Hb^- = hæmoglobin anions.
　　P = serumprotein.
　　P^- = serumprotein anions.
a, x, etc., are the concentrations.

The red corpuscle also contains other proteins besides hæmoglobin, but they are present in such small quantities that they may be neglected in an approximate calculation. Further in order to simplify the calculations, we shall assume that both the solutions are dilute and ideal or the simple theory of Donnan[4] can be applied to the system.

Hence

$$
\frac{(\text{Cl}^-)_1}{(\text{Cl}^-)_2} = \frac{(\text{HCO}_3^-)_1}{(\text{HCO}_3^-)_2} = \frac{(\text{Cl}^-)_1 + (\text{HCO}_3^-)_1}{(\text{Cl}^-)_2 + (\text{HCO}_3^-)_2} = r,
$$

where the brackets denote as usual the molar concentrations. With the same assumptions the conditions of the osmotic equilibrium give the following relation

$$
(\text{B}^+)_2 + (\text{Cl}^-)_2 + (\text{HCO}_3^-)_2 = (\text{B}^+)_1 + (\text{Cl}^-)_1 + (\text{HCO}_3^-)_1 + (\text{Hb})
$$

where (Hb) is the total concentration of hæmoglobin in the cell. These two relations give for r the following relation :

$$
r = \frac{b + c}{y + z} = 1 - \frac{a + (\text{Hb}) - x}{2(y + z)}
$$
$$
= 1 - \frac{a + (\text{Hb}) - x}{2\left[(\text{B}^+)_2 - x\right]}.
$$

A study of the chloride and bicarbonate distribution between plasma (venous) and spinal fluid, and plasma and ascitic fluid has been made by Muntwyler, Way and Pomerene[54] and they conclude that the

concentration and distribution of electrolytes in body fluids are governed by the factors outlined for the Donnan equilibrium. But their results as well as those of Hastings and others[55] show that $\frac{(\text{Cl}^-)_1}{(\text{Cl}^-)_2}$ is slightly less than $\frac{(\text{HCO}_3^-)_1}{(\text{HCO}_3^-)_2}$ which means that the conditions for the simple Donnan equilibrium are nearly but not exactly fulfilled. But this is what could be expected for in the simple theory we assume that both the solutions are very dilute and ideal, and we neglect the possibility of the formation of the ioncomplexes as well the adsorption of the ions. For a better agreement, more exact theory will have to be developed.

We have seen that the red corpuscles are not permeable by the cations, but Robinson and Hegnauer,[56] performed experiments with the plasma of cats and rabbits (which have received large intraperitoneal injections of isotonic glucose) and they conclude that when the electrolyte balance of the plasma is sufficiently altered, the red blood cell membrane may become somewhat permeable to cations.

Physico-Chemical Factors Affecting Intra-ocular Liquid.[57]

The earliest conception of the nature of the intra-ocular fluid was that it represented a secretion by the epithelium of the ciliary body, a conception based on insecure and inadequate evidence, mainly anatomical in nature. During the last thirty years there has existed alongside this view, the theory that the fluid in the anterior chamber was formed by simple filtration from the capillaries of the eye. Later physico-chemical researches of Duke-Elder[58] have given rise to the theory that this fluid is essentially a dialysate. We shall describe here the work of Duke-Elder[58] in detail.

The constituents of the serum are present in the intra-ocular fluid, these constituents may be divided into three groups of substances depending on the physical state of their molecules in solution, colloids, non-ionised and ionised crystalloids. All the substances

[54] Muntwyler, Way and Pomerene, *Journ. Biol. Chem.*, 1931, **92**, 733.

[55] Hastings, Sendroy, McIntosh and D'van Slyke, *ibid.*, 1928, **79**, 193.

[56] Robinson and Hegnauer, *ibid.*, 1936, **116**, 779.

[57] W. S. Duke-Elder, *Physiological Reviews*, 1934, **14**, 483.

[58] Duke-Elder, *Biochem. Journ.*, 1927, **21**, 66.

in colloidal aggregation are found in the intra-ocular fluid, but in much less concentration than in serum ; proteins, fats, immune bodies and ferments. All the proteins of the plasma are found in the intra-ocular fluid, they are found here in the same relative proportions in which they occur in blood, and they are specifically identical. They thus appear to remain unchanged in their transit from the blood-vessels to the eye. The diffusible non-dissociated substances are partitional between the intra-ocular fluid and the blood serum in approximately equal amounts. The more important constituents are sugar urea and the non-protein N. The dissociated diffusible substances are unequally distributed. In each case the cations have a partition coefficient > 1, and the anions a partition coefficient < 1, the former being in less and the latter in greater concentration in the intra-ocular fluid than in the serum. Cations are Na, K, Ca and Mg and the anions are Cl', SO_4'' and PO_4'''.

Duke-Elder finds that the osmotic pressure of the intra-ocular fluid is less than that of the capillary plasma by an amount determined by the excess of colloids in the latter. It is a small fraction only (about 0·3 to 0·5 per cent.) of the total osmotic pressure (approximately 6,000 mm. Hg).

Duke-Elder also studied the intra-ocular fluid formed under abnormal conditions :

(1) In the first series of experiments the permeability of the capillary walls is altered. He finds that when the permeability is increased very definite changes take place in the chemical and physical properties of the intra-ocular fluid, which are essentially the same no matter what method is employed to alter the permeability. The fluid so formed differs from the normal by approximating more nearly to the constitution of the plasma. The colloidal non-diffusible substances—proteins etc., are increased. The colloidal non-diffusible substances which are not icnised, such as sugar, are practically unchanged in concentration. The ionised salts are partitioned unequally ; the negatively charged anions show a diminution, and the positively charged cations an increased concentration. In a similar way physical properties vary in the direction an excess of colloids would lead us to expect.

(2) In the second method Duke-Elder altered the composition of the blood, either by varying the concentration of the normal constituents or by adding new substances to it. (This is of importance from the point of view of the local availability of drugs in the eye.) He finds that colloidal substances enter the eye, only in traces, if at all. Diffusible substances which are not ionised enter the eye freely, rapidly attaining therein a concentration equal to that in the plasma. Diffusible substances which are ionised behave in a manner depending on their electric charge. Anions—negatively charged substances like chlorides—readily pass into the eye, but cations do so only with difficulty.

Duke-Elder[59] analysed this experimental data, and assumed that the simple theory of Donnan may be applicable to this system. If Donnan's theory is applied the condition of equilibrium could be diagrammatically represented as follows :

Capillary blood.		Aqueous humour.	
	P		
Na^+	$\begin{cases} P^- \\ Cl^- \end{cases}$	Na^+	Cl^-
(1)		(2)	

where P = protein. This gives the theoretical relation

$$(Na^+)_{aq.} \times (Cl^-)_{aq.} = (Na^+)_{blood} \times (Cl^-)_{blood}.$$

Duke-Elder[59] found experimentally that L.H.S. $= 148·8$ and R.H.S $= 149·3$, which is a very satisfactory agreement with the theory.

Based on this experimental data, the theory of dialysation was elaborated by Duke-Elder ; that the intra-ocular fluid is in thermo-dynamical equilibrium with the capillary blood, and is a dialysate of it, the dialysing membrane being the capillary walls. It is thus comparable in its origin and metabolism with the tissue fluids elsewhere, and differs from them qualitatively only as a consequence of the relative impermeability of the capillaries of the eye. The capillaries vary in their permeability throughout the different tissues of the body, being adapted to suit the needs of each particular organ, and their almost complete impermeability in the eye may

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59 Duke-Elder, *The Nature of Intra-ocular Fluids*, London, 1927.

be regarded as a biological adaptation to keep the intra-ocular fluid as far as possible free from colloidal substances so that it remains optically homogeneous.

SWELLING OF PROTEIN GELS.[20]

The swelling of proteins is a subject of exceptional importance. The swelling of silk, cotton and wool fibres, of cellulose, and of various other plant and animal tissues, are the examples of this type of swelling. We have to deal with this type of swelling in the various manufacturing and biological processes.

A dry leaf of gelatin will, when placed in pure water, take in water and swell. This phenomena of imbibition-swelling can be prevented by applying pressure to the gel, and this equilibrium swelling-pressure may reach very high values when the gel contains only a relatively small proportion of the solvent. The nature of the intra-micellar or extra-micellar forces that determine this taking in of water are not yet fully understood. There exists, however, an apparently different sort of swelling, which we may call secondary or osmotic swelling. A piece of gelatine which has been fully swollen in pure water will show a further and much greater swelling in dilute acid or alkaline solutions. The forces concerned here are not great and a relatively small pressure is sufficient to prevent this type of protein swelling.

Procter and Wilson[60-62] consider this as a special case of Donnan's general theory of membrane equilibria. When gelatin is immersed in a dilute solution of an acid, combination takes place between gelatin molecules and the hydrogen ions, resulting in the formation of an highly ionisable salt of gelatin (formation of a non-diffusible gelatin cation), the anion of which is tending to diffuse, and exerts on the jelly mass an outward pull, which produces an increase in the volume of the jelly proportional to the magnitude of the pull. In pure water, combination must take place, although probably only to a very slight extent between the gelatin molecules and the hydrion of the slightly dissociated

water, leaving in the jelly a corresponding excess of hydroxyl ions which tend to diffuse outward causing the jelly to swell. According to the simple theory of Donnan the ionic products on each side of the jelly-water interface are equal, but the sums of the diffusible ions in the two systems are unequal. This inequality of the diffusible ions in the two systems causes an osmotic pressure in the jelly, which is greater than the osmotic pressure in the external fluid. This osmotic excess is the force which causes the jelly to swell, there is also a counterforce due to the elastic properties of the framework.

Procter carried out experiments to verify the theory. He assumed that the gelatin salt is completely ionised. If GH^+ represents the gelatin ions, *i.e.*, gelatin molecules combined with H^+, the state of equilibrium is represented diagrammatically as below :

Jelly.			External solution.	
z	G	H^+		
y		H^+	H^+	x
$y + z$		Cl^-	Cl^-	x

and $y (y + z) = z^2$. Procter found that this equation is satisfied qualitatively.

On the quantitative side the original Procter-Wilson theory gives only a rough approximation and requires modification in the light of later knowledge.[63] Procter-Wilson theory of gelatin swelling is based on the Donnan equations,[4] and these were worked out for diffusion into a volume sufficiently large, or for very dilute ideal solutions only. Experiments carried out on such systems show that as soon as structure begins to appear in a system, the simple calculations based on Donnan's equations[4] can no longer be applied in their unmodified form.

Donnan's original equations start with the assumptions that though the colloid ion is under mechanical restraint, all other ions and molecules are free from mechanical restraint. This hypothetical case is realised in a system where a membrane divides an entirely fluid system, *i.e.*, where the colloid is a sol, but as soon as we turn to consider gels, a different state of affairs make itself manifest. Ions and molecules can diffuse

60 H. R. Procter, *Journ. Chem. Soc.*, 1914, **105**, 313.

61 Procter and Wilson, *ibid.*, 1914, **109**, 307.

62 Wilson and Wilson, *Journ. Amer. Chem. Soc.*, 1918, **40**, 886.

63 D. Jordon-Lloyd, *Journ. International Soc. Leather Trades' Chemists*, 1933, **17**, 208.

through weak gelatin jellies as freely as through water. but as soon as the gel has a setting concentration of more than about 15% this is no longer the case, and by a time a 20% setting concentration is being considered, the hindrance to the migration of both water and dissolved substances is being considerable. In the tissue such as a muscle there is about 25% of protein and there is still a fair amount of freedom of movement for small molecules and swelling due to the establishment of Donnan equilibria takes place in acid and alkaline solutions. In skin where the protein concentration has increased to 35%, the freedom of movement of small molecules is still further reduced and the osmotic swelling is reduced accordingly. When we come to the group of fibres which in equilibrium with water contain 25 to 30% of water to 75 to 70% of protein, the structural arrangements of the long protein molecules must be such that nothing but long, fine capillaries remain for the small molecules to diffuse through, and the rate of diffusion is impeded. The mere crowding together of protein molecules leads to a reduction of the space available for free water and calls for the introduction of new and unknown factors into the simple equations.

IONIC NATURE OF ENZYMES.

The Donnan equilibrium furnishes a test for the ionic nature of any diffusible substance,[64] since the ratio of the concentration of any ion on the two sides of a membrane must be equal to the ratio of the concentrations of any other ion of the same sign and valence, whereas a non-ionic substance would be equally distributed on both sides. Expressed mathematically the equation is

$$\frac{(A_0^{-n})^{1/n}}{(A_i^{-n})^{1/n}} = \frac{(B_i^{+m})^{1/m}}{(B_0^{+m})^{1/m}} = \frac{(C_i^{+l})^{1/l}}{(C_0^{+l})^{1/l}}$$

in which (A_i) is the concentration of an n valent negative ion inside the membrane, (A_0) is the concentration of the same ion outside. B, C, etc., are any other diffusible ions present, having the valence m, l, etc. In order to test the ionic nature of a substance, therefore, it is only necessary to set up such an equilibrium system, measure the concentrations of some ion such as hydrogen or chloride and compare this

ratio with the concentration ratio of the substance under investigation. The only difficulty lies in the fact that the equation predicts only the concentration of the ions and not the total concentration, so that if the substance is not completely ionised, or is combined in non-ionic form in the solution, the determination of the total concentration will not lead to the correct ratio. In other words, if the experimental results do not agree with the ratio, the discrepancy may be due to complicating factors and no definite conclusion can be drawn, whereas if they do agree, the conclusion seems justified that the substance is ionic.

Northrop applied this method to the distribution of trypsin and found that trypsin behaves like a monovalent positive ion from pH 2 to 10·2.[64] At this point it behaves as though it were unionised and on the alkaline side of 10·2 becomes a monovalent negative ion. The experiments in this strongly alkaline range, however, are not satisfactory. Northrop[65] has also found that the ratio of the concentration of pepsin inside of gelatin or egg albumin particles to the concentration outside is approximately equal to the ratio of chloride or bromide ion under the same conditions. This is true over the range of pH from 1 to 7, and in the presence of various salts and acids. It follows, therefore, that pepsin is monovalent anion, and also that the enzyme does not form a compound with gelatin nor is the degree of dissociation affected by changes in pH in the range in which the enzyme is active. The enzyme becomes very unstable on the alkaline side of pH 7.

We shall now describe certain reactions which at least qualitatively can be explained on the basis of Donnan's theory. For example, it has long been known that dilute solutions of certain electrolytes will stimulate the action of enzymes. Falk[66] showed that dilute solutions of $MnSO_4$, $MnCl_2$, $MgSO_4$, $CaCl_2$ and $BaCl_2$ increased the activity of castor bean lipase toward ethyl butyrate. Calcium and magnesium salts accelerate tryptic digestion,[67] aluminium sulphate and monophosphates in dilute

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64 J. H. Northrop, *Journ. Gen. Physiol.*, 1924, **6**, 337.

65 Northrop, *ibid.*, 1925, **7**, 603.

66 Falk, *Journ. Amer. Chem. Soc.*, 1913, **35**, 601.

67 Cole, *Journ. Physiol.*, 1904, **30**, 202 and 281.

solution have been found to stimulate enzyme activity,[68] potassium bromate in low concentrations stimulates the digestion of casein by trypsin.[69] Not only is it possible to stimulate the activity of enzymes with salts but their presence is also capable of increasing enzyme production by microorganisms.[70]

In a series of papers dealing with the properties of an *antistaphylococcus* phage and its mode of action on a strain of *Staphylococcus aureus* Krueger[71] has shown that : In a mixture of phage and growing bacteria, phage in or on the cells is in equilibrium with the phage free in the medium. Phage formation is intimately related to bacterial growth and as far as can be determined does not occur in its absence. Phage can be completely inactivated by high concentrations of $HgCl_2$ and subsequently can be reactivated by removal of the Hg^{++} ions. Similarly inactivation with KCN can be reversed by conversion of the CN^- into $Ag (CN^-)_2$.

Krueger and West[71] performed experiments in connection with the accelerating effect of manganous salts on phage action. These experiments show that the accelerating effect is not due to a stimulation of bacterial growth nor to an enhancement of phage formation. There is a clear-cut lowering of the lytic threshold and also a change in the distribution of the phage between the bacterial cell and its environment. The Mn^{++} ion increases extracellular fraction at the expense of the phage fraction associated with the cell.

Padoa and Tedeshi[21] attempted to determine the membrane potential of oxidase and peroxidase (which are Fe and Mn containing the enzymes) in contact with an ionisable salt of the same ions. They find that under proper experimental conditions it is possible to determine at which pH the Mn^{++} ceases to act as a catalyst, it is between pH $6 \cdot 2$ and $6 \cdot 4$.

PHYSICO-CHEMISTRY OF CHEDDAR CHEESE MAKING.

McDowall and Dolby[72] find that there is a definite evidence of the existence of a Donnan equilibrium which controls the partition of ions between curd and whey and which also involves an osmotic effect which determines the concentration of non-electrolytes after salting. They show that after salting there is a pronounced fall in the concentration of calcium, a much less pronounced fall in phosphate and lactate, and a rise in sulphate in the wheys. With curd salted at an earlier stage than the normal, the whey contained a higher concentration of lactate/water, than did the whey from another portion of the same curd which had not been salted. Summing up these results it can be said that after salting there is an osmotic diffusion of water, from the curd tending to dilute all constitutents of the whey, while there is also a membrane effect which tends to increase the concentration of anions and decrease that of cations.

We can explain these results if we imagine a curd particle as being surrounded by a membrane permeable to all ions but protein ions, we obtain the conditions necessary for a Donnan equilibrium. It may be shown, theoretically, for such a case that the addition of an electrolyte such as sodium chloride will produce a rise in concentrations of cations in the outer liquid. The outward diffusion of water may be another result of this equilibrium or may be a simple osmotic diffusion due to the slow rate of diffusion of salt into the curd. The concentration of sodium chloride inside and outside the particle may be brought into equilibrium either by an outward diffusion of water or by an inward diffusion of sodium chloride. Until equilibrium is established, which may require some hours, both forms of diffusion will probably take place. Much of the water which diffuses out will escape as whey and will contain the various whey constituents in a more dilute form than prior to salting.

The Donnan equilibrium will have an important effect in determining the relation of the lactic acid in the curd to the whey acidity at all stages of the process. Any factors affecting this equilibrium will thus alter the significance of the whey acidities as a means of determining the extent of lactose fermentation in the curd. Among the factors controlling the equilibrium is the ionisation of the curd protein. This will be affected by the pH and also probably by other factors such as heat and rennet action, which alter the nature of the protein. It seems possible that in this equilibrium lies

[68] Schneidewind, *et al.*, *Landwirt. Jahrb. Schweiz.*, 1906, **35,** 911.
[69] Falk and Winslow, *Journ. Biol. Chem.*, 1918, **33,** 453.
[70] Robbins, *Amer. Journ. Bot.*, 1916, **3,** 234.
[71] Krueger and West, *Journ. Gen. Physiol.*, 1936 **19,** 75.
[72] McDowall and Dolby, *Journ. Dairy Research*, 1936, **7,** 156.

the key to the anomalous acidity readings mentioned by McDowall and Dolby.[72]

MEMBRANE EQUILIBRIA IN SOIL PHYSICS.

Lemmermann and Wiesmann[73] have found that the plants are better able to assimilate phosphoric acid from the soil, if the soil contains silicic acid, probably in colloidal form. Stollenwerk[73] working with Naaki observed that the manures in the form of potassium and phosphoric acid, can be used more economically when mixed with pure natural silicic acid. Silicic acid, SiO_2, which is negatively charged was of great help to the plants in the assimilation of food, whereas the positively charged iron hydroxide, $Fe(OH)_3$, hindered the assimilation of food. Stollenwerk[73] also carried out some experiments on marshy soil, making use of the solubility of the silicic acid in humic acid. It appears that the presence of the colloidal substances in the soils leads to a large yield, i.e., to a better assimilation of food. Applying the Donnan equilibrium to the process we see that the colloids which are undissociated and non-diffusible help the dissociated substances to diffuse into the plant. Stollenwerk's experiments also appear to indicate that singly charged cations are more easily taken in the plant in the presence of colloids than the doubly charged cations.

Similar experiments carried out by Behrens and Robertson[74] show that H ions are exchanged when neutral salts react with acid sphagnum peat. An equivalent entrance of the cation into the peat does not take place in this reaction. In some cases there is an increase in the cation concentration of the salt solution, in other cases there is an increase in the anion concentration. The influence of the concentration and the nature of the cations and anions upon the reaction suggested that the exchange might be explained on the basis of the establishment of Donnan equilibrium between the neutral salt solution and the acid peat.

COLLOIDAL PHENOMENA IN PLANTS

Colloidal systems involve small divisions of matter which expose large surfaces. The particles are, however, many times larger than the molecules so that ordinary molecular and ionic chemical reactions of the dissolved substances, are largely replaced by physical and chemical reactions dependent upon surface forces. A liquid colloidal system is a sol and a solid or a semisolid system a gel. The protoplasm of plants consists of colloidal systems varying from emulsions (sols) to moderately stiff gels. The solid substances of protoplasm proteins, dextrins, gums, etc., are characterised by the readiness with which they form colloidal systems in water.

The property[22] of not being permeable to substances dissolved in the cell sap is preserved by protoplasm only as long as it is alive. As soon as protoplasm dies, it loses its impermeability, and the substances dissolved in the cell sap diffuse out. The loss of impermeability by the killed cell results from changes in the state of the colloids of the protoplasm. From the condition of a sol they are transferred to that of a gel, the degree of their dispersion decreases, and their micellæ form irregular aggregates, between which, canals readily accessible to water and solutions are opened. This phenomena is called coagulation. The coagulation of protoplasm may be brought about by different causes, such as high temperatures, poisons, salts of heavy metal, acids, alkalies, as well as by an excessive withdrawal of water or even by mechanical pressure. In all cases coagulation leads to the death of the protoplasm. The phenomena of coagulation is, however, peculiar not only to protoplasm and albuminous substances in general, but also to other colloids of emulsoid and suspensoid character.

In many instances protoplasm may be dried to a state of complete air desiccation when usually 10 to 12 p. c. of the so-called hygroscopic moisture remains without losing its vitality, i.e., the faculty of returning to the sol condition. Thus seeds which in the ripening lose all free water and being air dry simultaneously suspend all vital processes, when moistened, once more return to activity. The same is true of many cryptograms, such as mosses, lichens, and some ferns. Not only their organs of reproduction but the whole thallus of these plants may be reduced to an air-dry condition. They return to activity when moistened. In such a dried protoplasm, therefore, some processes must be going on which alter the dispostion of its particles. When stored for a very long period, dry seeds lose their power of geminating. Under similar conditions dried cryptograms lose their capacity of returning to

73 W. Stollenwerk. *Zeit. Anorg. All. Chem.*, 1937, **231**, 192.

74 Behrens and Robertson, *Zeit. Pflanzenernähr, Düngung Bodenkunde*, 1931, **23 A**, 50.

life. Similar coagulation is observed when photographic plates are kept for a long time, their emulsion gradually becomes more and more coarse-grained and the plates lose their sensitivity to light.

All biological processes are characterised by life and the living cell is really a physico-chemical transformer which assimilates various substances and maintains itself in a state of dynamical equilibrium. Naturally many of the problems involving the application of colloidal physics and chemistry to protoplasma and plant physiology are but imperfectly understood. Still it has been possible to explain a large number of biological processes, at least qualitatively, by the application of Donnan equilibrium. Donnan considers an active living cell. In such a cell assimilation of substances oxidation and decomposition are taking place. The products of decomposition are excreted from the cell. In short the cell is continually changing its state. Now let us suppose that all the processes in the cell are stopped only for an instant. We are supposing that the cell is momentarily dead. To such a cell we can apply the laws of thermodynamics. Actually, how-

ever, the cell is continuously working. Our procedure in applying the laws of thermo-dynamics to the living cells is thus similar to the principle of virtual work.

The most essential facts about a living cell[75] are (a) its power of specific reproduction and repair, (b) its capacity for apparently purposive response and (c) its continual exchange of materials and energy. We know of no living organisms which remain indefinitely in a state of equilibrium without the liberation of energy. Life, in fact, is a self-perpetuating series of events : if the continuity of these events be broken by depriving them for a time of energy, their normal progress may be completely altered or prevented. We shall close this article by saying with Donnan.[76] ' Physical chemists were quite prepared to deal with stationary states—and have often done so—and would drop no tears if a rather naive thermodynamical treatment did not provide a complete explanation '.

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[75] A. V. Hill, *Trans. Farad. Soc.*, 1930, **26**, 667.

[76] F. G. Donnan, *ibid.*, 1930, **26**, 675.

An Automobile Factory in India.

AT the recent conference of the Congress Ministers for Industries held in New Delhi, Sir M. Visvesvaraya, who attended the meeting by special invitation, presented his important scheme for the manufacture of automobiles in India. India, in 1937, imported 16,036 cars and 13,046 commercial trucks, totalling 29,082 vehicles whose value is estimated at 8 crores. This is one of the ' Key ' industries which would pave the way for the manufacture of aeroplanes and armaments, so essential for the country's defence.

The scheme provides for the production of 10,000 cars and 5,000 trucks when the plant attains its maximum capacity. In the early stages, however, it is considered economical to import 30 per cent. of the special parts. 18 to 24 months would be required to put the factory into operation. In the first year, the factory will devote itself to assembling imported parts, some of which will be locally manufactured in the second year, and in the third year, the factory is expected to attain its full size and to manufacture the scheduled number of cars.

150 Lakhs is the proposed capital on

which a 20 per cent. return is expected. The Government of India's unstinted support by way of a high protective tariff and generous patronage, is essential for the promotion of the industry. Other progressive govern-ments in the West have allowed similar concessions, the import tariff levied in those countries ranging from 50 to 80 per cent.

The creation of an Automobile Industry in the country necessarily leads to the establishment of several other specialised industries. The car has some 2,000 separate parts and these are provided by the special factories. The Volvo Company of Sweden which manufactured a little over 6,000 cars in 1937, had contracts with 100 Swedish firms for the supply of parts. In India, this industry will use local steel and various other raw materials. It will thus help to develop mechanical skill of the highest order among Indian workmen. It will give employment to technically skilled young men. A factory like this established in the country will develop in our engineers and experts, capacity to design and operate high class machine industries of every kind including locomotives and aeroplanes,