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The formation and nature of mammalian cell membranes reconsidered

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The formation and functioning of mammalian cell membranes is reconsidered on the basis of the manner in which cells produce proteins, carbohydrates and other cell products. A description is given of formation, nature and operation of cell membranes which is compatible with known laws and observations of membrane interactions. The relationship to cell culture *in vitro* is considered.

Key words: mammalian cell membranes, formation, nature, stability, operation.

INTRODUCTION

Cell membranes perform several functions including isolation of cell activities, control and transport of chemical reactants (inorganic and organic) into the cell and control and release of cell reaction products from the cell. The means whereby membranes perform these operations has been the subject of study for decades and has given rise to numerous hypotheses (Skon, 1957; Post *et al*., 1972; Singer & Nicholson, 1972; Lux, 1979; Shen *et al*., 1986; Stein, 1986; Dai & Sheetz, 1999) which are based on the physical and chemical properties of amphiphilic lipid molecules (Imberg, 2003). It is possible to envisage that cell formation occurs as a sphere composed of one or more of the components of a membrane which expands to the final cell size. Such a situation means that the membrane components and cell products are initially formed outside of the cell and the formation transfers to the inside of the membrane at some unspecified points of time in cell development. The alternative is that cell formation is initiated by reactions giving rise to two or more of the membrane components. The latter enclose the former allowing the continuation of the initiating reactions and developments of these reactions. The components of membranes are lipids, proteins, carbohydrates, glycerol molecules and long chain carboxylic acids. The principle reactions in biosystems are hydration and dehydration in the forming and breaking of peptide and glycosidic bonds, dehydration in the formation of esters, reduction, oxidation, release of carbon dioxide from the carboxylic acid group (decarboxylation) and the removal and decomposition of amino groups (deamination). The spatial arrangement of groups of organic compounds is also involved in these reactions. It follows that, to form the components of membranes, an effective dehydration agent is present in cells. None of the reactants or products of the above reactions can act in this context and the agent required is not known. The study below advances a model of the formation and nature of cell membranes.

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THE NATURE OF BIOLOGICAL FLUIDS

The presence of low concentrations (millimoles per litre) of ionic compounds which are normally highly soluble in aqueous media (Table 1) in the intracellular (cytoplasm) and intercellular fluids is indicative of a nature different from pure water (Ling, 2004). The viscosity of the fluids is higher than water further indicating a difference from pure water. Proteins and carbohydrates readily form hydrophilic sols. The colloidal material in a hydrophilic sol can also be caused to precipitate as a gelatinous solid by reducing the temperature of the sol. The solids so formed can be

reconverted into a sol by increasing the temperature. This property differentiates a sol from an emulsion. It is known that cells can be inactivated by freezing and reactivated by return to body temperature (37°C) supporting the nature of the fluid involved as a sol. All sols are sensitive to the the presence of ionised electrolytes. The presence of small amounts of charged particles derived from ionised electrolytes in a hydrophilic sol has little effect. Increased amounts cause the colloidal particles to be precipitated as gelatinous solids. The ion concentrations required to cause precipitation are millimoles per litre in keeping with the low ion concentrations above. Reduction of the ion concentration results in the gelatinous solids redissolving. Different sols also vary in stability with respect to charge density. The reactions above will also be favoured by taking place in a hydrophilic sol which can be considered as a semi-solid. This restricts molecular motion (rotation, vibration) to a greater extent than a free liquid allowing molecular spatial position and orientation to become effective factors in cell reactions.

THE FORMATION OF BIOLOGICAL MEMBRANES

The ionic strengths of the intracellular and intercellular fluids in the human metabolism are shown in Table 1. The concentration of the ionic species in both fluids is low enough to allow the assumption that the activity coefficients are unity. The results show that the ionic strength of the intracellular fluid greatly exceeds that of the intercellular fluid indicating that the two sols are different in stability towards high charge concentrations. The intercellular sol being more readily precipitated by a given concentration of charge than is the case for the intracellular sol. Mutual precipitation on contact of sols with opposite charge or different concentrations of the same charge

TABLE 1. The ionic strengths of human intracellular and intercellular fluids (data from Braun, 1999)

	mol l^{-1}	**Ionic charge**	**Ionic strength**	**Total ionic strength**
Intercellular cations				
Na^+	0.140	0.140	0.1000	**0.2865**
K^+	0.004	0.004		
Mg^{2+}	0.004	0.016		
Ca^{2+}	0.010	0.040		
Organic Acid	0.006	0.006		
Protein	0.012	Non ionised		
Intercellular anions				
Cl^-	0.105	0.105	0.1840	
HCO_3^-	0.050	0.200		
PO_4^{2-}	0.006	0.054		
SO_4^{2-}	0.002	0.008		
Intracellular cations				
Na^+	0.010	0.010	0.1920	**1.5315**
K^+	0.150	0.150		
Mg^{2+}	0.050	0.200		
Ca^{2+}	0.006	0.024		
Organic Acid	0.008	0.008		
Protein	0.055	Non ionized		
Intracellular anions				
Cl^-	0.002	0.002	1.3395	
HCO_3^-	0.008	0.032		
PO_4^{2-}	0.285	2.565		
SO_4^{2-}	0.050	0.200		

type is a general phenomenon. The membrane is formed by precipitation of the components from the intercellular sol at the boundary between the intracellular sol and the intercellular sol. A cell will remain stable as long as the internal high concentration of charge is maintained and any of the intercellular sol making contact with the membrane will precipitate on this surface. The intracellular sol in contact with the inner surface membrane requires a greater charge density than the intercellular sol to cause precipitation. Contact of intracellular sol with the membrane will result in the areas of contact dissolving. Part of the latter material undergoes cell chemical reactions giving rise to the cell products. Some of the dissolved material and the reaction products from the higher charge regions in the intracellular sol are precipitated to give the internal components of cells.

An effective dehydration/hydration agent present in cells is polyphosphoric acid. This acid exists in various spatial forms (shown in Fig. 1) and undergoes reversible hydration and dehydration to monophosphoric acid. Polyphosphates have been shown to be present in cells (Grossmann & Lang, 1962). It has been shown that in mixtures of water and phosphorus

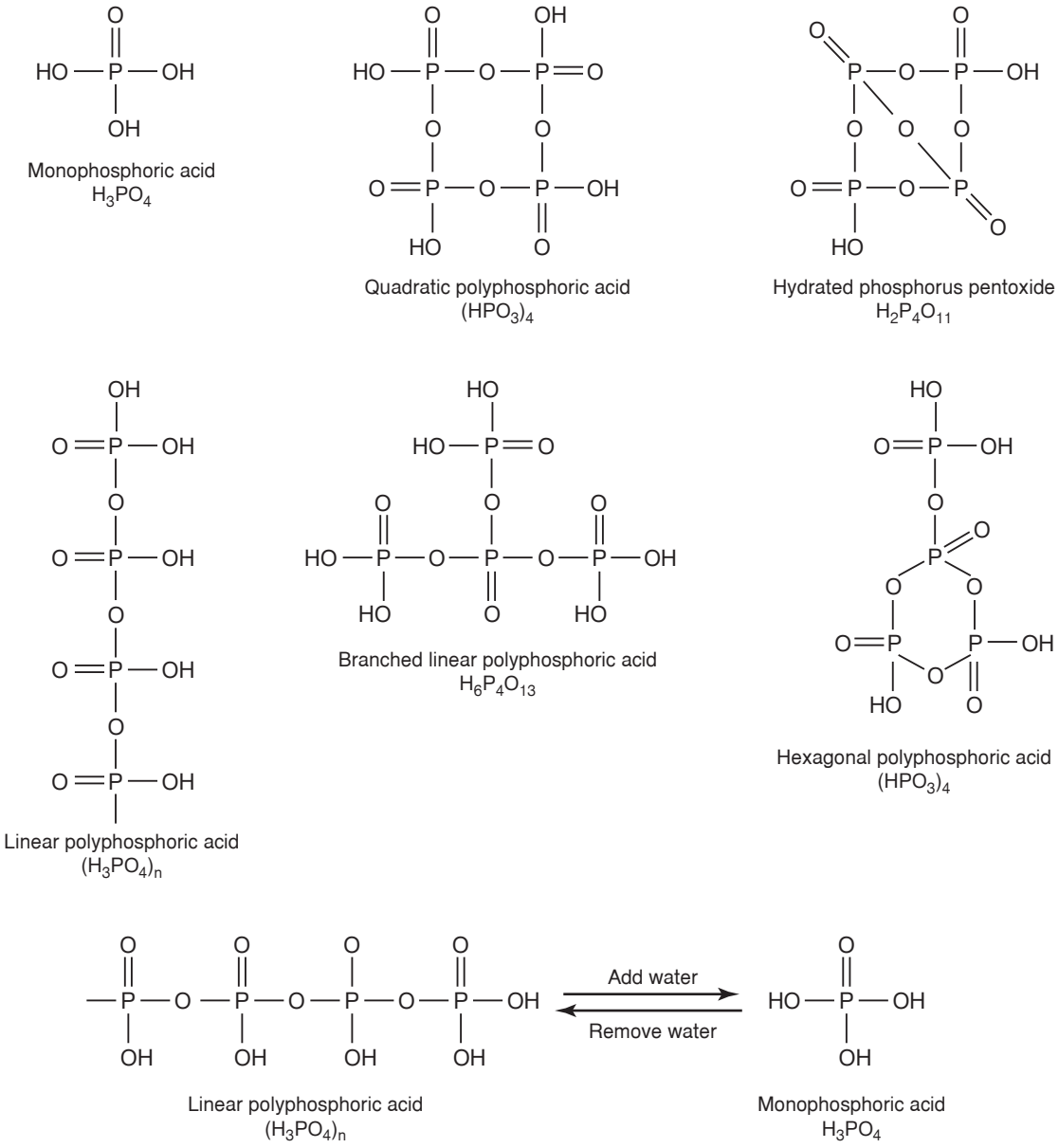

FIG. 1. The forms of polyphosphoric acid.

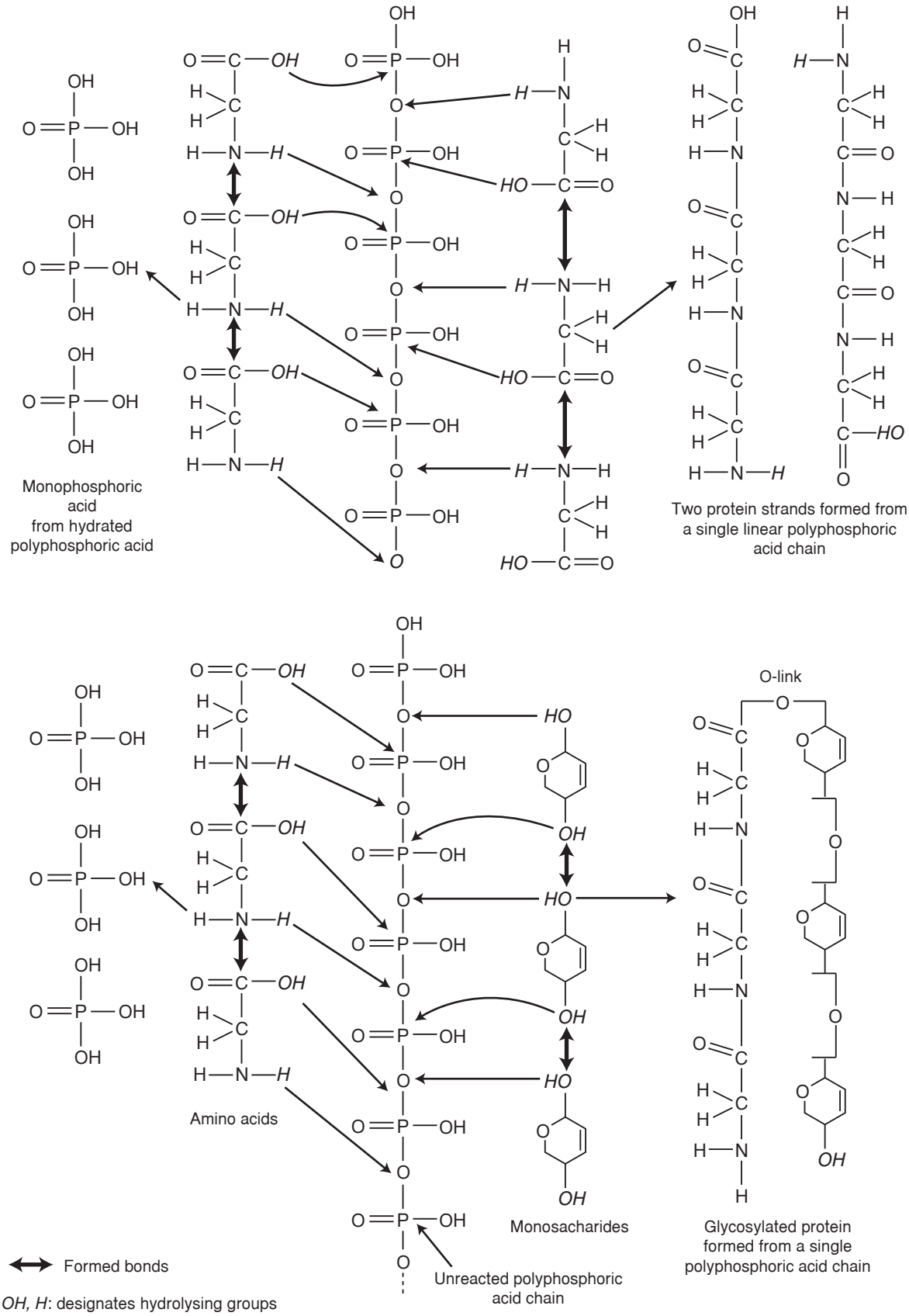

FIG. 2. The formation of proteins.

pentoxide the various forms of polyphosphoric acid are dominant when the molecular ratio of these two compounds is one to one (Slack, 1968).

This is in keeping with the nature of the fluid in cells where the free water content is low. The conditions in a given fluid (pH value, temperature, nature and concentration of cations present) are the factors which determine whether monophosphoric or polyphosphoric acid is the stable form (Slack, 1968). When the former is the stable form, hydrolysis of proteins occurs giving amino acids and polyphosphoric acid. When the latter is the stable form, dehydration of amino acids occurs giving proteins and monophosphoric acid. Metabolic reactions involving these acids are examples of stereochemical reactions which involve the stereochemistry of an inorganic molecule as well as of organic molecules. The dehydration of amino acids by linear polyphosphoric acid giving rise to proteins and monophosphoric acid is shown in Fig. 2. Lipids are formed in a similar manner where the polyphosphoric acid removes the water of esterification supporting the latter reaction. The amino acids can also be linked in a similar manner as shown giving rise to lipids like lecithin which contains an amino group. The combination of the above ionic conditions and hydration/dehydration reactions gives rise to a membrane structure of the type shown in Fig. 3.

As demonstrated, hydrogen and hydroxyl ions are displaced by the dehydration action of polyphosphoric acid molecules within the membrane. These hydrogen-hydroxyl displacements form ion bridges linking the membrane units together and allow expansion and contraction of the membrane surface. Polyphos-

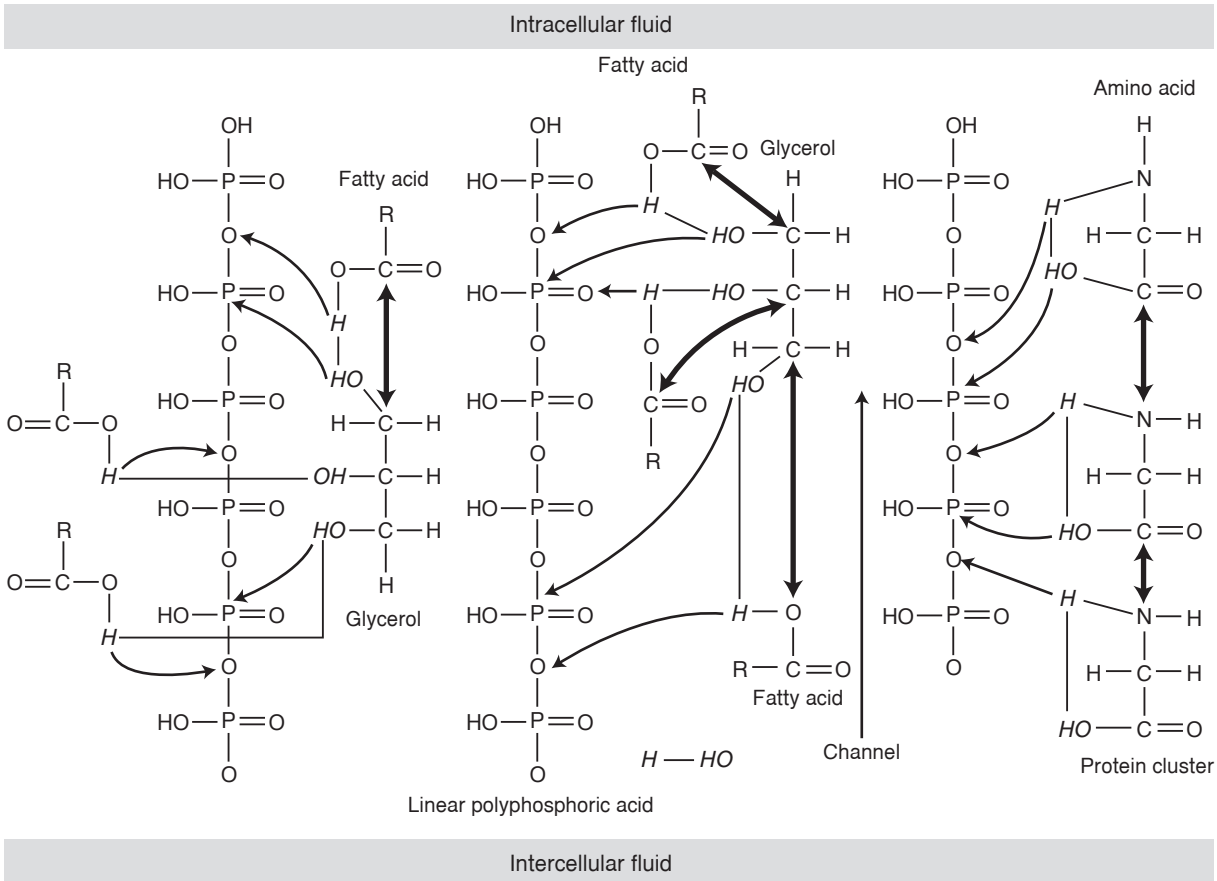

FIG. 3. The construction of a cell membrane.

phoric acid molecules within the membrane and within the cell are formed by water leaving the membrane and the cell by osmosis or electro-osmosis. This results in dehydration of monophosphoric acid molecules entering the membrane from the intercellular fluid and within the cell. Ion concentration differences and charge differences exist across the membrane, as shown in Table 1. The concentration of sodium ions outside the cell is very similar to the concentration of potassium ions inside the cell and there is little sodium in the cell and little potassium outside the cell. The concentration differences and electrochemical potentials of these ions (sodium, –2.711 V, potassium, –2.924 V) give rise to a potential difference known as the membrane potential which has a value of the order of 70 mV. This results in water leaving the cell by electro-osmosis resulting in a cyclic process for the formation of polyphosphoric acids. As shown in Fig. 3, channels exist in the membrane as the result of its structure. The channels contain the minimum number of hydrogen-hydroxyl ion bridges. A dominance of either of these ions in a given channel results in the channels being charged either positively or negatively and the channels are the source of the measured membrane current (Neher & Sakmann, 1976). The effect of continuous precipitation and dissolving processes described is that the intracellular fluid is continuously advancing towards the boundary with intercellular fluid. The position in the fluids where the rate of dissolving and the rate of precipitation are in equilibrium, is the point at which the membrane is formed. The three-layered structure of membranes observed in electron micrographs consists of an outer diffusion dominated layer, a central zone containing the partially formed compounds shown in Fig. 4 and an inner dissolving zone.

The deposition of compounds on the exterior surface of a cell membrane is controlled by the presence of the diffusion dominated layer. This is a static boundary layer of fluid several microns thick. Molecular and ionic motion in this layer is affected by Fick's Law, the Soret Effect and the membrane potential. As precipitation proceeds the ions and compounds in the diffusion layer fluid will be partitioned between the central zone and the intercellular fluid. The result is that concentration gradients are formed in the diffusion dominated layer as shown in Fig. 4. The thickness of the diffusion dominated layer is related to the dimensions of the cell involved. The operations in this layer will remain undisturbed by the movement of the fluid leaving the cell through the channels in the membrane or by the movement of the cell as a whole through the intercellular fluid. Only compounds present in this layer affect the ionic strength of the fluid which results in the precipitation of compounds at the outer surface of the membrane.

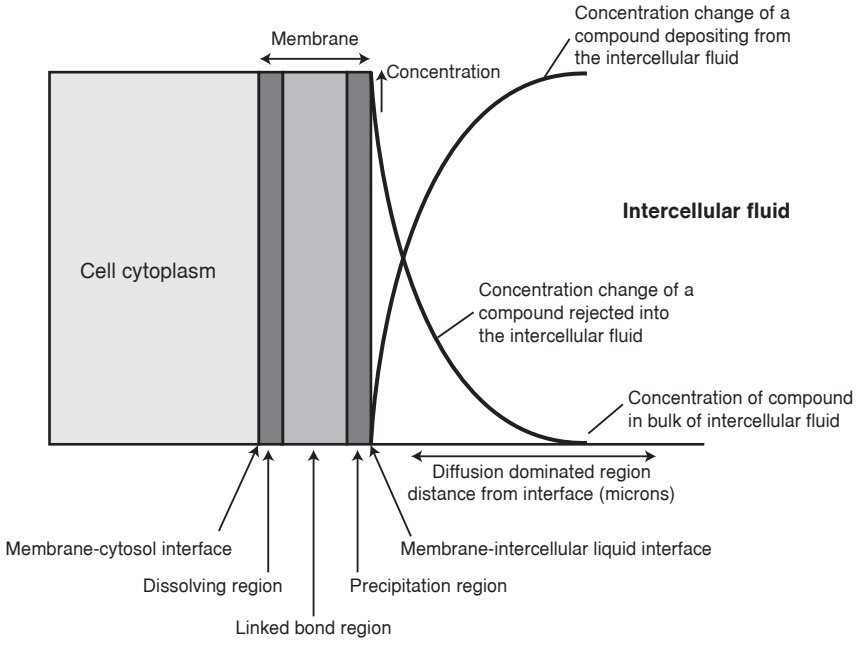

FIG. 4. Concentration variation in a diffusion dominated layer.

Ionic compounds trapped by co-precipitation at the external surface of the membrane are carried through the membrane and enter the cell by the continuous dissolving of the innermost surface of the membrane. The amount of any ionic compound trapped and transported is related to the molecular nature, concentration and solubility of the compound in both the intercellular fluid and the deposit. For example, the K^+ ion is transferred into the cell by one or more potassium compounds being trapped by precipitation at the outer surface of the central zone and leaves the cell by diffusion through the charged channels as an ion under a concentration gradient. For the Na^+ ion, the situation is reversed and the ion enters the cell by diffusion under a concentration gradient through the charged channels and leaves the cell as a component of cell products such as the sodium salt of carboxylic acids. This difference being the result of solubility differences in the relevant compounds present in the intercellular sol. Insoluble suspended particles in the intercellular fluids (virus capsules, carbon microgranules) can be trapped by deposition at the external surface of the membrane and carried through the membrane into the cell by the continuous dissolving of the innermost surface of the membrane. Any compounds produced by other cells can also enter a given cell by deposition on the membrane surface and physical transport though the membrane.

For cell reactions to continue the chemical compounds required for the reaction have to reach the reaction zone and the chemicals produced by the reaction have to be removed from the reaction zone. As cell products are continuously formed, then the cell volume will increase and the surface area of the membrane will be expanded. This expansion will be opposed by a force of contraction generated by the ionic bridges within the membrane. This results in a pressure inside the cell being higher than that in the intercellular fluid as is the case of an oil droplet in water. The pressure results in liquid products being extruded into the intercellular fluid through the channels. As cell compounds are formed and leave the cell, the compound components leave the inner surface of the membrane maintaining the volume and composition of the cytoplasm constant. The precise chemical composition of a cell membrane is indicative of the nature of the chemical compounds dissolved in the intercellular fluid in contact with any group of cells. It follows that the chemical composition of the membranes of a particular group of cells does not need to be the same as any other group.

MEMBRANE STABILITY

The stability of cell membranes is sensitive to changes in ionic strength of the fluid in which the cells are suspended. Membranes of cells can be stable with respect to one fluid and unstable with respect to the second fluid. Designating the ionic strength of fluid of the diffusion dominated layer as μ_1 and that of the fluid normally in contact with the diffusion dominated layer of a cell membrane as μ_2 then for cell membrane stability $\mu_1 > \mu_2$. Cell membranes are therefore liable to be affected under conditions, where μ_2 changes value or is replaced by a fluid with a different value, designated as μ_3. These changes being permanent or intermitent. In the event that a cell passes into a fluid or is subjected to a fluid with an ionic strenght $\mu_3 < \mu_2 < \mu_1$ then the cell membrane will be stable, as is the case for μ_2. When $\mu_3 = \mu_2 < \mu_1$ the cell membrane will still be stable. However, when $\mu_3 > \mu_2$ and $\mu_3 > \mu_1$ then membrane becomes unstable and disintegrates. An unstable membrane can be stabilised as the result of changes in the ionic strength of the intercellular fluid arising from interaction with fluid from the cell reducing μ_3 to less than or equal to μ_2. The membrane of cells from one part of the metabolism will not necessarily survive in the fluid of another part of the metabolism. On this basis, cells from a part of the metabolism can interact with and destabilise cells from another part of the metabolism. Compounds formed in a cell do not lower the ionic strength of the fluid outside on leaving the cell to a point where membrane disintegration would occur. This only occurs when the cell products are altered by abnormal cell activity such as is caused by bacterial cell or virus capsule interaction with a cell. Membrane disintegration can also occur when abnormal cell activity in a remote part of the metabolism results in a reduction of the ionic strength of the fluid reaching particular cells.

EXPERIMENTAL EVIDENCE SUPPORTING THE MODEL

The media used to culture cells *in vitro* have the same function as the intercellular fluid in the metabolism. The ionic strength of Ham's culture medium shown in Table 2 is less than that of the intercellular fluid. The products of the cells *in vitro* will change this value in time. This situation is unlike the operations of the metabolism in which the ionic strength of the intercellular fluid at any particular part of the metabolism is maintained constant by transfer of components

TABLE 2. The ionic strength of Ham's cell culture medium

Compound	Molecular Weight (gms)	Weight used (gms l^{-1})	Mols l^{-1}	Ionic charge	Total ionic strength
Na_2HPO_4	358.14	0.2900	8×10^{-4}	0.0097	0.1943
KH_2PO_4	136.09	0.0830	6×10^{-4}	0.0073	
NH_4Cl	53.49				
NaCl	58.44	7.4000	0.1266	0.2532	
$MgSO_4$	120.37				
$CaCl_2$	110.99	0.0440	3×10^{-4}	0.0023	
$MgSO_4{\cdot}7H_2O$	246.48	0.1530	6×10^{-4}	0.0049	
$FeSO_4{\cdot}7H_2O$	278.05	0.0080	2.9×10^{-6}	2.3×10^{-5}	
$CuSO_4{\cdot}5H_2O$	249.68	$2.5x10^{-6}$	1×10^{-8}	8×10^{-8}	
KCl	74.56	0.2850	3.82×10^{-3}	0.0076	
Arginine	174.20	0.2110	0.0012	0.0012	
Histidine	155.16	0.0210	1.3×10^{-4}	1.3×10^{-4}	
Lysine	146.21	0.0293	2×10^{-4}	2×10^{-4}	
Methionine	149.21	0.0044	3×10^{-5}	3×10^{-5}	
Phenyalanine	165.19	0.0045	3×10^{-5}	3×10^{-5}	
Tryptophan	204.23	0.0006	2.9×10^{-6}	2.9×10^{-6}	
Tyrosine	181.19	0.0018	9.9×10^{-6}	9.9×10^{-6}	
Alanine	89.10	0.0089	1×10^{-4}	1×10^{-4}	
Glycine	75.07	0.0075	1×10^{-4}	1×10^{-4}	
Serine	105.10	0.0105	9×10^{-5}	9×10^{-5}	
Threonine	119.12	0.0035	2.9×10^{-5}	2.9×10^{-5}	
Aspartic acid	133.11	13.3000	0.099917	0.099917	
Glutamic acid	147.13	0.0147	9.9×10^{-5}	9.9×10^{-5}	
Aspargine	132.13	0.0150	1.1×10^{-4}	1.1×10^{-4}	
Glutamine	146.13	0.1452	9.9×10^{-4}	9.9×10^{-4}	
Isoleucine	131.17	0.0026	1×10^{-5}	1×10^{-5}	
Leucine	131.18	0.0131	9×10^{-5}	9×10^{-5}	
Proline	113.13	0.0115	9.9×10^{-5}	9.9×10^{-5}	
Valine	117.15	0.0035	2.9×10^{-5}	2.9×10^{-5}	
Cysteine	204.30	0.0315	1.3×10^{-4}	1.3×10^{-4}	

from the blood. Most mammalian cells fail to grow in this and other culture media unless some blood serum is present. It is presently considered that adding serum to a cell culture medium provides the cells with growth factors. The latter consist of a wide range of proteins which have been observed to support, and in some cases interfere with, cell division in both the metabolism and *in vitro*. Within the metabolism, growth factors are the reaction products of cells. This being the case, the addition of the factor, produced by particular cells, to an *in vitro* culture of the same cells will slow or stop the cell reaction producing the growth factor and therefore reduce cell activity. In addition, different serum batches with identical ionic strength vary in their ability to support cell growth. On the basis of the above description, the addition of blood serum supplies the proteins necessary to form a sol analogous to the intercellular fluid. However, the added proteins should not be those formed by the cells being cultured. In addition, the culturing media must have the same ionic strength as the intercellular fluid in the part of the metabolism where the cells are situated. The precipitation of gelatinous solids from a hydrophilic sol (destabilisation of the sol) has long been known to be dependent on the nature of the ionic species which form a series known as the lyotropic series. Ions in the series are present in mammalian metabolisms. The cations in the series in order of effectiveness are $Mg^{2+} > Ca^{2+} > Na^{+} > K^{+}$ and the anions are citrate 3^{+} > tartrate $3^{+} > SO_4^{2+} > C_2H_3O_2^{-} > Cl^{-} > NO_3^{-} > I^{-}$. This means that as cell growth proceeds *in vitro* any increase in the

concentration of these ions as the result of cell operations will lead to destabilisation of the *in vitro* sol leading to a decrease and eventually cessation of cell operations. This situation would be avoided by a continuous flow of a fresh culture medium of the correct ionic strength analogous to blood flow. The culture of mammalian cells is found to be effective only at body temperature (37˚C) indicating that the cell sol is sensitive to temperature, as described above.

CONCLUSIONS

Cell membranes are dynamic by nature being continuously formed at one surface and continuously dissolved at the second surface. The above mechanism means that the stability of cells in any metabolism is a function of the nature of fluid and the ionic strength of the fluid. A cell membrane will become unstable and will disintegrate when the nature of the intercellular fluid is altered such that this sol becomes more stable against ion concentration. In conditions where the ionic strength of this sol increases or can be increased to a value equal to or greater than that which gave rise to the precipitation of the cell membrane, the latter will dissolve. Changing the nature of the intracellular fluid such that this sol becomes less stable against ion concentration, will result in cessation of the internal dissolving process described. The result will be conversion of the entire cell to a gelatinous solid. Under these conditions, the cell will cease to function. This mechanism becomes important in the interaction of bacteria and viruses with mammalian cells. The presence of the diffusion dominated layer of the same composition and ionic strength, is the reason living cells not to coalesce on contact. The observed phenomena of engulfing of one cell by another arises as the result of membrane penetration of one cell by another under conditions where ionic strength of the fluid between two cells leads to dissolving of the parts of each cell membrane resulting in coalescence of cytoplasmic fluids.

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