Early Osmotic History of the Plasma Membrane

By MERKEL H. JACOBS, PH.D.

The following topics, among others, are considered: Pfeffer's hypothesis of an invisible plasma membrane, to account for similarities in the osmotic behavior of plant cells and artificial osmometers; early doubts as to the reality of Pfeffer's membrane; denial by colloid chemists of the existence of plasma membranes because of the supposed failure of cells to obey osmotic laws; demonstrations of thin, external cell membranes by electrical and micro-manipulative methods; use of the plasma membrane in the mathematical treatment of cell permeability; the nature of so-called 'perfect osmometers'; the erythrocyte as an osmometer; hemolytic evidence of the existence of an erythrocyte membrane.

THROUGHOUT its entire history, the plasma membrane concept has been closely associated with osmotic phenomena. It was the similarity of behavior of plant cells and artificial osmometers that led W. Pfeffer to postulate for the former, invisible Plasma-membranen, having properties resembling those of his artificial copper ferrocyanide membranes. Following the publication in 1877 of his Osmotische Untersuchungen,1 three periods, each roughly a quarter of a century in length (with some overlapping), can be distinguished. During the first, important osmotic studies were made, but without convincing proof or even general acceptance of Pfeffer's concept. The second period was characterized, on the one hand, by attacks on the plasma membrane by colloid chemists who believed that cells do not obey osmotic laws; on the other, by convincing objective evidence that the surface of a cell differs fundamentally in its physical properties from the internal protoplasm. During the third period the, by then, generally accepted plasma membrane was made the basis for the quantitative treatment of cell permeability, and previous arguments based upon supposed osmotic abnormalities of cells were in many cases shown to be invalid. More modern developments, including refinements of experimental methods and mathematical procedures and recent important studies of active transport and of the behavior of artificial membranes, fall outside the scope of this brief résumé of early osmotic history.

Early Doubts

Pfeffer's hypothetical membrane was—to use his own word for it—merely a Notbehelf which he said he would gladly abandon if the evidence compelled him to do so. He then proceeded experimentally to examine the evidence and published much of it in 1890.2 The results were, on the whole, favorable to his theory and almost, but not quite, convinced him of its correctness. In the second edition of his Pflanzenphysiologie,3 published in 1897, he admitted that a layer of protoplasm of appropriate properties but of almost any thickness could conceivably perform the osmotic functions of his postulated invisible membrane.

It is of interest to note that Pfeffer was primarily concerned with pressure changes in plant cells at nearly constant volume. The result was the early appearance of the term osmotic pressure. Had his studies been made with mammalian erythrocytes, in which a delicate biconcave membrane offers almost no resistance to expansion, the important generalizations of van't Hoff would have been delayed; but certain common misconceptions concerning the nature of osmotic pressure might have been avoided.

Unlike Pfeffer who was interested in the entrance of water into plant cells at almost constant volume, de Vries,4 who followed him and also used plant cells, studied the escape from them of water at nearly constant pressure. The plasmolytic method enabled him
to determine the isotonic coefficients for electrolytes, on which the theory of electrolytic dissociation of Arrhenius was in part founded. Strangely enough, though de Vries\(^5\) made experimental studies of the membranes that surrounded the internal sap vacuoles of plant cells, he did not believe that a plasma membrane at the cell surface was responsible for his osmotic results; these he attributed to the properties of the protoplasm as a whole.

A few years later Hamburger,\(^6\) in the first serious osmotic study of an animal cell (the mammalian erythrocyte), obtained isotonic coefficients that agreed well with those of de Vries, but neither at this time nor later in his monumental three-volume *Osmotischer Druck und Ionentheorie*\(^7\) did he appear to consider the possible existence of a plasma membrane.

The next important step was the use of osmotic volume changes as a measure of the rate of entrance of solutes into living cells. Of fundamental importance in this field was the work of Overton,\(^8\) who in 1899 was able to report that during the preceding nine years, in some 10,000 experiments, he had studied the behavior of over 500 different chemical compounds. He arranged these substances in classes according to their respective rates of entrance into cells and made important generalizations as to the physical and chemical properties of molecules that favor rapid entrance. His best known generalization concerns what is now commonly called lipid solubility. It still plays an important part in any serious discussion of the plasma membrane, though Overton himself preferred to speak merely of an "impregnation" of the cell surface with substances such as lecithin and cholesterol, which he believed also to be present in the cell interior. Extensions of his generalizations to the mammalian erythrocyte were made by Grynszpan\(^9\) and Hedin,\(^10\) using dissimilar osmotic methods, but neither of these workers seems to have felt the need of a plasma membrane to explain his results.

**Colloid-Chemical Objections**

A quarter of a century after the introduction of Pfeffer's plasma membrane concept there was still no convincing argument either for or against it. The most unfavorable facts were that such a membrane had never been seen and that no other satisfactory evidence had yet been given that there is a fundamental difference between the surface layer and the interior of a cell. Both of these objections were soon to be removed, but only after the membrane theory had been subjected to a severe attack by colloid chemists to whom the colloidal behavior of protoplasm provided an all-sufficient explanation of biological phenomena. According to M. H. Fischer: "There are no membranes about cells. All the phenomena which are so difficult to explain when we assume a membrane to exist about cells are readily interpreted without recourse to such postulates on the basis of the colloid constitution of protoplasm."\(^11\)

Since the position of the colloid chemists was essentially negative, they had some difficulty in finding direct positive evidence to support it, but even such evidence as they had was used with poor logic. A common fallacy appears in the reasoning of Lepeschkin,\(^12\) which was as follows: Cells are not surrounded by solid precipitation membranes. Neither are they surrounded by liquid layers of fatty material brought to their surfaces by physical forces. Therefore, they have no membranes at all, and their behavior must depend upon the *einheitlich* nature of their colloidal protoplasm. To many persons in those days an explanation of cellular behavior, to be scientific, had to be of a very simple physicochemical character. The possibility that the plasma membrane might be a part—even a complex part—of the cell itself seems to have been disregarded, possibly because such a belief was thought to be too vitalistic.

The fear of complexity is well illustrated by another quotation from Fischer.\(^11\) Apparently wishing to eliminate the plasma membrane from consideration by a *reductio ad absurdum*, he said: "From the original osmotic membranes of Pfeffer which were semi-permeable, we have come to those which are partially permeable and then to those which are permeable sometimes and then again are..."
not,''' and which (he added in substance) "are permeable not only to lipoids but to salts and water as well." Actually this reductio ad absurdum could pass for a modern description of a plasma membrane, except that it is too simple.

Early Positive Evidence

The downfall of the colloid-chemical point of view was brought about by the objective demonstration in several ways that there is at the surface of a cell a very real, thin layer having physical properties strikingly different from those of the cell interior. In several papers, the first of which appeared in 1910, Höber13 showed by different methods that while erythrocytes in their entirety have an extremely high electrical resistance, the interior of an intact cell is a good conductor. Later workers, by measurements of the very high electrical capacity of erythrocytes and other cells, showed that the almost nonconducting surface layer must be extremely thin. A discussion of the early history of this important field, with references to the literature, is given by Höber,13 who, it should be mentioned, in successive editions of his admirable Physikalische Chemie der Zelle und der Gewebe did much to ensure a favorable reception of the plasma membrane concept. Of particular historical importance in the objective establishment of the reality of the plasma membrane were the micromanipulation studies of Chambers, which for the first time gave direct visual evidence of the existence of such a membrane. Earlier workers had tried in various ways to demonstrate a miscibility of protoplasm with water but had failed because of the rapidity with which cells can repair or establish a new membrane. Chambers14 developed for the first time a technic by which a membrane-free drop of water or of an aqueous solution could be introduced into the interior of a cell. Under these conditions a free and rapid diffusion of both water and solutes was found to occur within the cell boundaries. By the use of solutes which were either already visible or which could be made so by their effects on vitally stained cells, it was found that a substance which fails to cross the surface of the cell in either direction may diffuse freely within its interior. Other equally convincing demonstrations of the reality of the plasma membrane have been devised and discussed at length by Chambers.15, 16

Permeability Coefficients

With the general acceptance of the plasma membrane concept, a simple mathematical treatment of the kinetics of water movements between cells and their surroundings become possible. Assuming the membrane to be very thin and to account for all but a negligible part of the resistance of the system to the flow of water, equation 1 provides an approximate measure \((P_W)\) of the rate of movement of water under standardized conditions between an external solution of a nonpermeating solute of osmotic concentration \(C_M\) and a cell. This equation in various forms has been much used (for early examples see Lucké and McCutcheon17):

\[
\frac{dV}{dt} = P_W A \left( \frac{C_o V_o}{V} - C_M \right) \tag{1}
\]

The use of a permeability coefficient \((P)\) to define permeability to a solute depends upon the same assumptions as regards the plasma membrane but is complicated by the fact that in animal cells osmotic changes of \(V\) nearly always accompany movements of a solute, \(S\). The general case involving movements of both water and a solute is approximately described by the simultaneous equations 2 and 3 (see further Jacobs18, 19, 20). For reasons discussed by Staverman,21 a permeating solute does not have the osmotic effectiveness predicted from the freezing-point depressions of its solutions, but for practical purposes, particularly when the permeability of the cell to \(S\) is not too great, equations 2 and 3 in many different forms have been frequently and advantageously employed, e.g., Wilbrandt.22, 23

\[
\frac{dS}{dt} = PA \left( C_s - \frac{S}{V} \right) \tag{2}
\]

\[
\frac{dV}{dt} = P_W A \left( \frac{C_o V_o}{V} - C_s - C_M \right) \tag{3}
\]
Equation 2 is particularly useful in the special case where $V$ has the constant value $V_0$ and $A$ is also constant. Direct integration is then possible, and the resulting equation has been much used in work with radioactive isotopes. Very important historically was the use of the same equation by Collander and Bärlund in the determination of many permeability coefficients for cells of the plant Chara. Their results gave valuable information about the properties of plasma membranes, confirming in an accurate quantitative way the Overton principle of lipid solubility and showing in addition the influence of the molecular volume of a hydrophilic solute upon its rate of passage across a cellular membrane.

Similar comparisons by others of the permeability of homologous cells of different species to the same solute or solutes give striking evidence of characteristic species differences, with accompanying evidences of biological relationship. Such results confirm the belief, already arrived at on other grounds, that the plasma membrane is an integral and complex part of the cell itself.

Studies of permeability also give evidence that the part of the cell which regulates the rate of uptake of solutes is at or very close to its surface. For example, the normally rapid rates of entrance of glycerol into, and escape from, human erythrocytes are greatly slowed by traces of copper in the external solution. Copper is known not to enter erythrocytes under these conditions, and the same is true of proteins which can reverse its effects, yet both the effect and its reversal are almost instantaneous. Tannic acid in dilute solutions likewise fails to enter erythrocytes, but its presence in the external medium can rapidly and reversibly retard the movement of anions across the cell surface.

Osmotic Volume Changes

The behavior of the cell as an osmometer has frequently entered into discussions of the plasma membrane, the assumption being made that "imperfect" osmotic behavior indicates either the absence or some abnormality of such a membrane. Neither of these conclusions is justified. The plasma membrane and the true osmotic behavior of a cell might both be "perfect" and yet the cell be a "bad" osmometer in the sense of showing an inconstant CV product, $C$ being the osmolar concentration of the external solution and $V$ the volume of the cell water.

The implied definition of a perfect osmometer tacitly assumes two things, neither of which may be true: (1) that the pressure within the osmometer remains constant; and (2) that there is no change in the number $(N)$ of osmolar equivalents of internal solutes. A more satisfactory definition of a perfect osmometer would be that for equilibrium at constant pressure and temperature, $N/V = C$, or otherwise written, $CV = N$. It happens that for most animal cells $N$ is reasonably constant; but for reasons to be discussed below this is not true of the mammalian erythrocyte, which has in consequence a bad reputation as an osmometer. But it by no means follows that it has either a "bad" membrane or none at all.

A favorite argument of the colloid chemists against the existence of a plasma membrane was based upon the belief that (to quote one of them) "today we may safely say that we do not know of a single cell for which the laws of osmotic pressure are valid." Such an argument in effect employs the following syllogism: (a) a perfect osmometer with a perfect membrane shows a constant CV product; (b) no cell shows a constant CV product; (c) therefore cells do not possess plasma membranes.

This is very bad logic. Obviously there are other reasons than the absence of a plasma membrane why a cell might fail to show a constant CV product. For example, (1) such a membrane might be present but (a) be leaky or (b) offer resistance to expansion; or (2) a perfect membrane might be present but free expansion of the cell be prevented and pressure created (a) by external restriction, as in a plant cell, or (b) by the physical properties of the protoplasm, or, further, (3) a membrane might be present and free expansion of the cell be possible, but $N$ have a variable
value because of (a) changes of internal stoichiometric concentrations or (b) of osmotic coefficients or (c) both.

The Erythrocyte

The behavior of the mammalian erythrocyte as a CV osmometer is notoriously irregular. According to Ponder,31 "the conclusion that the mammalian red cell is a perfect or even a good osmometer is, for example, incredible." In addition to the inconstancy of its CV product, the erythrocyte shows other apparent osmotic anomalies. For example, it has long been known that it tends to have a smaller volume in a solution of a nonpermeating nonelectrolyte, such as sucrose, than in one of NaCl having the same freezing point. This peculiarity is the basis of an interesting experiment in which the addition of a drop of saturated NaCl to a suspension of red cells in a solution of sucrose causes the cells to swell rather than to shrink.32 Here the osmotic effect appears to be "wrong" not merely in degree but in direction. Even worse is a case of what at first sight seems to be a completely erratic and unpredictable type of osmotic behavior (which nevertheless has an entirely reasonable explanation).33

Despite these and other apparent abnormalities of the erythrocyte, there is no reason to believe either that its true osmotic behavior is anything but normal or to resort to colloidal explanations which deprive it of a membrane. The fact is that for the red cell at osmotic equilibrium, just as for other cells, N/V = C. The difference is that, in most cells, N is reasonably constant, while, in the erythrocyte, N normally changes its value twice during each circuit of blood through the body and can readily be changed artificially in vitro in accordance with well-understood principles. If, under these circumstances, despite a change in the value of N, the CV product were found to remain constant, then the cell could truly be said to show "bad" osmotic behavior.

The tendency of N to change is closely related to the hemoglobin which forms about one-third of the weight of the human erythrocyte. In the first place, according to Adair and others, the osmotic coefficient of hemoglobin changes far more rapidly with changing concentration than those of most other solutes; this is particularly true at high concentrations, i.e., low values of V. Changes in the value of C may therefore produce disproportionately great changes in the value of N. The result is a smaller change in V than would otherwise be needed to preserve the equality of N/V with C. This effect, however, is not due to any abnormality in the true osmotic behavior of the cell—and least of all to any peculiarity of its membrane—but merely to a well-known property of hemoglobin.

The hemoglobin of the red cell also has more complex and far-reaching indirect effects on the distribution of water. These are described in principle in figure 1, which represents some aspects of a much simplified, hypothetical erythrocyte and is largely self-explanatory. The points of greatest importance for the present discussion are a "proton pool" (H+) shared by several chemical systems, a free permeability to small anions, CO2

Figure 1

A much simplified representation of some of the ionic equilibria within the erythrocyte which influence its volume under various changing conditions.
and water, with virtual impermeability (over short periods) to K\(^+\) and Na\(^+\) and also to Hb. At body reaction, Hb carries many negative charges, of which for simplicity only one is here represented. Moderate changes in the number of these charges have little direct osmotic effect; their indirect effect, however, is very important. When, by way of the proton pool and the anion-permeable membrane, a reduction of the negative charges on the hemoglobin results in an increase in the sum of the HCO\(_3^-\) and Cl\(^-\) ions in the cell water, the entirely orthodox osmotic result would be an increase of cell volume.

Among the common factors affecting the volumes of erythrocytes in this direction are a rise of CO\(_2\) tension, addition of ionized HCl to the external medium (with an exchange of OH\(^-\) from the cell for Cl\(^-\) from the medium), "washing" erythrocytes in a neutral, isosmotic salt solution with a resulting exchange of external Cl\(^-\) for partially replaced HCO\(_3^-\), lowering the temperature, and reduction of oxyhemoglobin. The reversal of all these treatments would of course cause shrinkage. Further details will be found elsewhere.\(^{34, 35, 37}\)

The free permeability of the erythrocyte to anions is responsible for its apparently bizarre behavior in solutions of nonelectrolytes. With an anion-permeable membrane, a cell could lose some of its own ions in exchange for hydroxyl ions from the external medium, where they are always present in minute but constantly replaceable quantities. The OH\(^-\) ions are largely neutralized within the cell, with an increase of the negative charges on the hemoglobin, while the sum of the internal Cl\(^-\) and HCO\(_3^-\) ions is decreased, and the cell in consequence shrinks. Supporting this theory of the mechanism of shrinkage is the fact, first noted by Coulter\(^{36}\) and confirmed by later observers, that under these conditions dissociated HCl appears in the nonelectrolyte solution.

As for such peculiar osmotic behavior as increased swelling in a more concentrated solution, the agreement of observation with theory is better than merely qualitative. A discussion of the osmotic volume changes of cells having different types of permeability in mixtures of electrolytes and nonelectrolytes gives further details.\(^{37}\) Some previously unpublished calculations of theoretical equilibrium volumes of variously treated erythrocytes in different mixtures of sucrose and NaCl are represented in figure 2. Actual tests have shown good semiquantitative agreement with all these predictions; in particular, the added NaCl-total solute ratios for the maximum volume, and for recovery of the initial volume, are remarkably close to the theoretical ones. Not only does this superficially puzzling behavior of the erythrocyte fail to indicate the absence of a cell membrane, but it requires a membrane of well-known properties for its explanation.

Ionic Permeability

Positive evidence that the erythrocyte is surrounded by a plasma membrane is provided by a comparison of the theoretical and observed osmotic effects, discussed more thoroughly elsewhere,\(^{37}\) of pH changes on the volumes of cells having three types of ionic permeability: (I) cells freely permeable to anions but not (or only very slowly) to cations; (II) cells impermeable to both kinds of ions; and (III) cells freely permeable to both. Type I is usually believed to be represented by the normal erythrocyte; type II can be approximately obtained by treatment of erythrocytes by very low concentrations of tannic acid, and type III, by their exposure to an appropriate concentration of butanol. All three types are permeable to water and impermeable to hemoglobin.

The theoretical behavior of cells of these various types can be illustrated by a much simplified system containing internally NaCl and an ionized Na salt of hemoglobin, and externally NaCl at a constant osmolar concentration of 2C\(_o\). The pH-dependent Donnan ratio for anions, represented by r, has a value of unity at pH 6.8, the isoelectric point of oxyhemoglobin, and progressively decreases with increasing alkalinity. The usual simplifying assumptions for approximate calculations have been used in obtaining the conditions for simultaneous osmotic and ionic
equilibrium shown in the bottom spaces of table 1.

It will be noted that, for cells of type I, the water volumes should decrease with increasing alkalinity. This is the behavior always observed with normal erythrocytes. Type II cells should assume a finite equilibrium volume which is pH independent. Normal erythrocytes do not behave in this way. Type III cells should swell indefinitely (and therefore undergo hemolysis) at all pH values, but theoretically they should do so most slowly at the isoelectric point of hemoglobin where \((r + 1/r)\) is equal to 2 and the only osmotic unbalance is due to the relatively small contribution of the hemoglobin. This unbalance could, if desired, be removed by the addition externally of a nonpermeating solute, such as sucrose. It is very clear that normal erythrocytes, which shrink rather than swell with increasing alkalinity, do not fall in type III and therefore do not behave as if they lacked membranes.

An application of these principles to butanol-treated erythrocytes was made by Netsky and Jacobs, who found that, as demanded

<table>
<thead>
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<th>Type</th>
<th>II</th>
<th>I</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeable to</td>
<td>H₂O</td>
<td>H₂O, Cl</td>
<td>H₂O, Cl, Na</td>
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<tr>
<td>External concentration</td>
<td>2C₄</td>
<td>2C₄</td>
<td>2C₄</td>
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<tr>
<td>Internal concentration</td>
<td>Hb + Na + Cl</td>
<td>Hb + Na + rC₄</td>
<td>Hb w – (r + 1/r)C₄</td>
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<tr>
<td>Equilibrium value of w</td>
<td>2C₄</td>
<td>(2 – r) C₄</td>
<td>∞</td>
</tr>
</tbody>
</table>

Figure 2

Theoretical volumes of erythrocyte water after different additions (in isosmotic units) of NaCl to an originally isosmotic solution of sucrose in which the cells are initially suspended. The permeability types are those mentioned in the text.

Table 1

Theoretical Ionic and Osmotic Equilibria of Erythrocytes of the Three Permeability Types Mentioned in the Text
A previously unpublished experiment by Dr. M. G. Netsky, showing the protection of beef erythrocytes against butanol hemolysis at pH values near the isoelectric point of hemoglobin.

References

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