Early Osmotic History of the Plasma Membrane

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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 resumé 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 biconeave 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

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to determine the *isotonic coefficients* for electrolytes, on which the theory of electrolytic dissociation of Arrhenius was in part founded. Strangely enough, though de Vries⁵ 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 Tonoplaste⁷* 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 Gryms⁹ 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 colloidal 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 physiochemical 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.¹³ 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.
Höber\textsuperscript{13} showed by different methods
that while erythrocytes in their entirety have an
extremely high electrical resistance, the in-
terior 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 noncon-
ducting surface layer must be extremely thin.
A discussion of the early history of this im-
portant field, with references to the litera-
ture, is given by Höber,\textsuperscript{13} who, it should be
mentioned, in successive editions of his ad-
mirable \textit{Physikalische Chemie der Zelle und
der Geweben} 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 micromanipula-
tion studies of Chambers, which for the first
time gave direct visual evidence of the exist-
ence 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.
Chambers\textsuperscript{14} developed for the first time a
technic by which a membrane-free drop of
water or of an aqueous solution could be in-
troduced 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 sub-
stance 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.\textsuperscript{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 approxi-
mate measure ($P_\omega$) of the rate of move-
ment of water under standardized condi-
tions between an external solution of a nonper-
meating solute of osmotic concentration $C_M$
and a cell. This equation in various forms has
been much used (for early examples see Lucké
and McCutcheon\textsuperscript{17}):

$$\frac{dV}{dt} = P_\omega A \left( \frac{C_v V}{V} - C_M \right) \quad (1)$$

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 move-
ments of both water and a solute is approxi-
mately described by the simultaneous equa-
tions 2 and 3 (see further Jacobs\textsuperscript{18, 19, 20}).
For reasons discussed by Staverman,\textsuperscript{21} a per-
meating solute does not have the osmotic effec-
tiveness 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 fre-
quently and advantageously employed, e.g.,
Wilbrandt.\textsuperscript{22, 23}

$$\frac{dS}{dt} = P A \left( \frac{C_v - S}{V} \right) \quad (2)$$

$$\frac{dV}{dt} = P_\omega A \left( \frac{C_v V}{V} - C_v - C_M \right) \quad (3)$$
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\(^2\) 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.\(^{25-28}\) 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.\(^{29}\) 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.\(^{30}\)

**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 osmolal 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 osmolal 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)\(^{11}\) "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.
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 hemo- 

globin results in an increase in the sum of the HC₀₃⁻ and Cl⁻ ions in the cell water, the entire 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 CO₂ 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, isos- 
motic salt solution with a resulting exchange of external Cl⁻ for partially replaced HC₀₃⁻, lowering the temperature, and reduction of oxyhemoglobin. The reversal of all these treatments would of course cause shrinkage. Further details will be found elsewhere. 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 ca-
tions; (II) cells impermeable to both kinds of ions; and (III) cells freely permeable to both. Type I is usually believed to be repre-

sented 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 2C₀. 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 per-
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

\[
\begin{array}{ccc}
\text{Type} & \text{II} & \text{I} & \text{III} \\
\text{Permeable to} & H_2O & H_2O, Cl & H_2O, Cl, Na \\
\text{External concentration} & 2C_a & 2C_a & 2C_a \\
\text{Internal concentration} & \frac{Hb + Na + Cl}{w} & \frac{Hb + Na}{w} + rC_a & \frac{Hb}{w} + \frac{(r + 1/r)C_a}{2} \\
\text{Equilibrium value of } w & \frac{Hb + Na + Cl}{2C_a} & \frac{Hb + Na}{(2 - r)C_a} & \infty
\end{array}
\]
by the theory for cation-permeable cells, osmotic swelling and hemolysis in an isosmotic NaCl solution occur over a wide pH range with a minimum rate at about pH 6.8, the isoelectric point of hemoglobin; this pH was the same for eight different species of mammals. Furthermore, protection against the butanol effect was given by the presence in the external solution of nonelectrolytes of high molecular weight. This was the first convincing evidence for a mechanism of hemolysis involving the Donnan equilibrium, independently suggested by several persons and called the "dual mechanism" by Davson and Ponder[39] and "colloid-osmotic" hemolysis by Wilbrandt,[40] who first recognized its wide occurrence and practical importance. Figure 3, previously unpublished, represents a typical experiment made by Dr. Martin Netsky with beef erythrocytes.

**Figure 3**
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.

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References


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