A MECHANISM FOR THE TRANSFER OF WATER AGAINST AN OSMOTIC GRADIENT, WITH SUGGESTED BIOLOGICAL IMPLICATIONS[1]

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It has long been known that living cells, in certain glands, are capable of performing osmotic work. The mammalian kidney, for example, can form urine having an osmotic pressure much higher than that of the blood. It is known that the energy for this work is derived from intra-cellular chemical reactions; but the *mechanism* by which cells transfer water against steep gradients of osmotic pressure remains a mystery. In purely physical systems osmotic work may be carried out by the application of mechanical pressure, by means of distillation,[2] or by the aid of electro-osmotic forces. It is possible that all three processes occur, to a greater or lesser degree in living tissues, but attempts to account for the secretion of water on the basis of these forces have not proved entirely satisfactory.

There is, however, a simple mechanism for the performance of osmotic work which does not appear to have been considered by those who have studied secretion; nor has it been described in the literature of physical chemistry, so far as the author is aware. It will be shown that, under certain conditions, osmotic and hydrostatic forces alone can suffice to transfer water from a concentrated to a dilute solution; and it is suggested that the physical conditions for such a transfer may be present in the cells of living organisms.

The mode of operation of an internal osmotic gradient to transfer water against an external gradient of osmotic concentration. A cell model has been constructed in this laboratory which operates successfully to transfer water from a 20 per cent solution of sucrose, in one chamber, to distilled water in another, at constant temperature. The transfer continues for hours at the rate of approximately 0.1 cc. per minute, which, in relation to the dimensions of the membranes (diameter 10 cm.; thickness, 0.05 mm.), is sufficiently rapid to preclude any doubt as to the validity of the observations. Attempts to stop the process by setting up an opposing difference in hydrostatic

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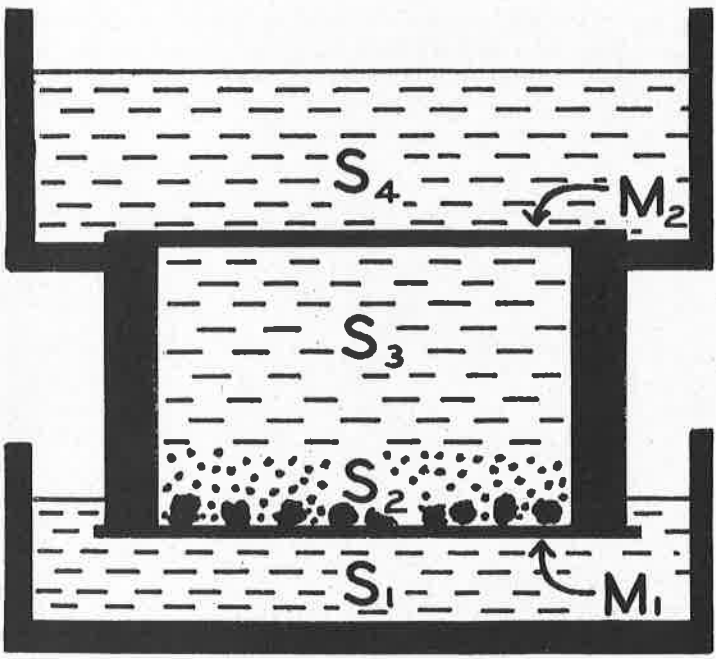
[2]A solution having an osmotic pressure of 60 atmospheres at 38°C. will have a vapor pressure which is lower than that of the pure solvent (water) by only 2.2 mm. Hg. Raising the temperature of the solution 1°C. will increase its vapor pressure 2.6 mm. Hg. Hence, at 39°C. the solution has a lower "osmotic pressure" than its solvent at 38°C., and water will distill from solution to solvent.

pressure of 400 mm. Hg (the upper limit of pressure tolerated by the membranes) have failed; the fluid transfer, though somewhat retarded, continues steadily.

A diagram representing the essential features of the model is shown in figure 1. In the actual apparatus all chambers are closed except at the openings of three capillary side-tubes (1.5 mm. bore) which facilitate observations of the direction and rate of movement of water and of the pressure relationships in the various chambers. A 20 per cent solution of cane sugar, s_1, is placed in the lower chamber, in contact with the collodion membrane, m_1, which is relatively impermeable



to sugar. The central chamber is filled first with distilled water. Ordinary table sugar is then poured into the chamber to a depth of about 0.5 cm. above m_1. The sugar forms a saturated solution above m_1, but due to the slow rate of diffusion and the higher density of the concentrated solution the concentration of sugar at the top of the cell, at s_3, remains very dilute for many hours; in one experiment the concentration at s_3 was only 0.5 per cent at the end of 5 hours. A second membrane, m_2, separates the cell from the chamber of distilled water, s_4. The osmotic pressure of s_1 is approximately 15 atmospheres, but that of s_2 is much higher—probably at least 100 atmospheres—so that water passes from s_1 to s_2. This osmotic transfer of water raises the hydrostatic pressure within the cell, and as soon as the

pressure becomes higher than the difference in osmotic pressure between s_3 and s_4—in this cell the difference is negligible—water begins to pass from s_3 to s_4. In the case under consideration, or in any case where the osmotic pressure of s_4 is equal to that of s_3, water is filtered (or ultrafiltered) from s_3 to s_4 as soon as the intracellular pressure rises above that of the atmosphere. The pressure will continue to rise so long as water comes into the artificial cell by osmosis faster than it can be filtered out. The rising hydrostatic pressure facilitates filtration and retards osmosis, until eventually a steady state is reached at which the rates of passage of water through m_1 and m_2 are equal.

Assuming equal effectiveness of hydrostatic and osmotic pressure differences to produce movement of fluid, and assuming that m_1 and m_2 offer equal resistance to the passage of water, and where h is the final steady pressure within the cell and s_1, s_2, s_3, and s_4 represent the osmotic pressures of the various solutions, it is evident that $h - (s_3 - s_4) = (s_2 - s_1) - h$; whence, $h = \frac{(s_3 - s_4) + (s_2 - s_1)}{2}$. In the case under consideration, where $s_3 = s_4$, $h = \frac{s_2 - s_1}{2} = \frac{100 - 15}{2} =$ 42.5. If the membranes were strictly semipermeable, the intracellular pressure would rise to 42.5 atmospheres. The cell can operate at atmospheric pressure throughout (at the steady state) only when $(s_3 - s_4) + (s_2 - s_1) = 0$, or when $s_4 - s_3 = s_2 - s_1$; that is to say, when the osmotic pressure within the cell at one end is as much below that of the outer solution, s_4, as the osmotic pressure at the other end of the cell exceeds that of the outer solution, s_1. On the other hand, if $s_4 - s_3$ exceeds $s_2 - s_1$, the value of h will be negative, so that at the steady state the intracellular pressure will be less than atmospheric.

For purpose of analogy with systems of living cells, it will be most profitable to consider the conditions which give rise to the smallest possible positive values of h. It is probable that in such systems one will have to deal with cases in which s_3 is approximately equal to s_4. In such cases, provided the membranes offer equal resistances to the passage of fluid, h will have a small positive value provided s_2 is only slightly greater than s_1. However, it is possible to have s_2 much greater than s_1 if the membrane m_1 offers much more resistance to the passage of water than does m_2. Let the ratio, $\frac{\text{resistance of } m_1}{\text{resistance of } m_2} = r$. Then for cases where $s_3 = s_4$; $h = \frac{s_2 - s_1}{1 + r}$; for strictly semipermeable membranes. Hence, as r increases without limit the value of h will approach zero—atmospheric pressure—for any positive value of $s_2 - s_1$. If the membranes are permeable to some or all of the solutes, the value of h will be further reduced. If the cell, instead of being rigid, is readily distended by the incoming water, the pressure

at any moment will be determined in part by the volume-elasticity of the cell; the cell may continue to swell during the early stages of the withdrawal of water from s_1, but eventually, as s_1 becomes more concentrated—or more generally as $s_2 - s_1$ decreases—the intracellular pressure will fall and the cell volume will decrease. In such cases the internal pressure may never reach a steady state.

It is evident that the model cell can be made to transport water against a high external gradient without developing a significant increase in internal pressure, provided the volume-elasticity of the cell, the resistances of the membranes, and the gradients of osmotic pressure across the membranes are properly adjusted. Eventually, of course, the internal gradient will level off, as the result of the diffusion of sugar from s_2 to s_3, and as the result of the disappearance of solid sugar by dissolution. The cell will then require a "rest," i. e., work will have to be done to restore the initial intracellular gradient. It would be possible, in theory, to drive the cell continuously by introducing some chemical mechanism into the region of s_3 which would transform dissolved sugar to solid sugar. The solid sugar would drop to the bottom and enable the cell to continue working; but energy would have to be supplied from the outside to drive the chemical reaction: we are not dealing with a perpetual motion machine.

An osmotic pump operating directly through the cooperation of osmotic and hydrostatic forces in the manner described above would seem to offer the simplest possible hypothesis to explain certain secretory processes of living cells. It is only necessary to assume that these cells can develop and maintain for some time in a steady state an intracellular osmotic gradient. The region of highest concentration of dissolved material may perhaps be confined to a narrow zone adjacent to the membrane at one end of the cell, so that the *average* concentration of the protoplasm need not be significantly higher than that of the surrounding fluids.

Evidence for the existence of osmotic gradients in living cells. Actual proof of the existence of effective osmotic gradients within the cells of various tissues must await further investigation, but the evidence at hand indicates that gradients of metabolic activity, which in general are known to be associated with differences in osmotic pressure, probably do occur in gland cells. The fact that gland cells capable of doing osmotic work are invariably oriented with respect to their blood supply on the one side and with the gland duct on the other is presumptive evidence that metabolic gradients occur in such cells. The basal end of each gland cell is close to the blood capillaries and therefore may be presumed to have a higher oxygen tension than the peripheral end of the same cell, which may be expected to suffer, at times, from a deficiency of oxygen.

At low oxygen tension the oxidative recovery processes of cells are reduced and dissociative reactions lead to the accumulation of osmotically active break-down products such as lactic and phosphoric acids: a paramecium placed in a fluid which is deficient in oxygen will swell

and finally burst, indicating that the osmotic pressure of its protoplasm has been markedly increased. Even in the presence of oxygen, acceleration of functional activity of a cell, such as is brought on by specific stimulation, may lead to the rapid accumulation of osmotically active metabolic products: during severe exercise lactic acid is formed so rapidly in muscle cells that the osmotic pressure of the blood may increase 10 per cent above the normal level (Hill, 1931); a fatigued muscle swells when placed in an originally isotonic solution. It may therefore be stated as a reasonable hypothesis that the osmotic pressure in any cell or part of a cell will be increased when the metabolic ratio, $\frac{\text{rate of cellular dissociative activity}}{\text{rate of oxidative recovery}}$, is increased. It does not seem impossible that the value of the metabolic ratio may vary considerably in different parts of the same cell.

Steep gradients of tension of the gases, oxygen and carbon dioxide, have been observed to occur in tissues. Gases from the intestine, under certain conditions, have been reported to contain oxygen at a tension so low that it was not measurable (McIver, Redfield, and Benedict, 1926) and this suggests that a steep gradient of oxygen tension may be set up within the epithelial cells of the intestinal mucosa. A carbon dioxide tension of 200 mm. Hg has been found in certain samples of human urine (Sendroy, Seelig, and Van Slyke, 1934). Correspondingly high tensions of this gas have been found in the intestinal juice of dogs (deBeer, Johnston, and Wilson, 1935). Carbon dioxide diffuses so rapidly in tissues that the finding of a drop in tension from 200 mm. Hg on one side of an epithelial membrane to perhaps 40-50 mm. in the venous blood on the other side compels one to believe that steep concentration gradients, not only of gases, but also of other, less diffusible materials, may commonly occur within the cells.

The gradient theory as applied to the transfer of dissolved substances. It is evident that, for the study of secretion, the concept of intracellular metabolic gradients offers a possible explanation of the power of certain glands to perform osmotic work. Such work may involve the transfer of water in the manner described above. It may also include the transport of dissolved substances against diffusion gradients of these materials. For if any substance is rapidly changed or broken down at one end of a cell and rapidly reformed at the other end, that substance must diffuse into the first end from the external medium, there to break down; the products will of necessity diffuse to the other end of the cell—provided they are incapable of escaping through the cell membrane—and there the original material will be reformed. Here the material will continue to accumulate until its concentration exceeds that of the external fluid, when it will diffuse out of the cell. In this way, by the operation of a metabolic gradient, any substance may in theory be completely removed from the fluid

bathing one surface of a glandular tissue and be transported to the fluid on the other side of the living membrane.

It is probable that the chemical reactions involved in the excretory or secretory transport of materials by living cells are those very chemical reactions which are necessary for the ordinary life processes of the cell. It is probable, also, that the apparently purposeful regulation of the transfer of materials by certain tissues is due to the more or less direct effects of environmental changes upon these *ordinary* but *spatially oriented* intracellular chemical and physical processes. According to the gradient hypothesis, then, one must study the selective transfer of any substance by seeking first for the optimum conditions for the chemical change or breakdown of the material; secondly, one must find the conditions which favor resynthesis or freeing of the material in its original state; then one must seek a transition within the excretory cells from one of these chemical conditions to the other, sufficiently steep and oriented in the proper direction to account for the process of transfer; and, finally, one must show how the "excretory gradient" is brought into play as a result of the accumulation of an excess of the material in the cell or its environment. Since the ability of certain cells to regulate the fluid composition of their environment involves also the process of retention of *needed* materials at optimum concentrations we must also study the selective absorption and conservation of such materials according to the same general principle, *viz.*, that the harmful change in concentration brings into play a gradient of chemical activity directed in such a way as to restore the concentration of each substance to the normal value, either by causing selective excretion or selective absorption of the material in question.

Summary

1. An experiment is described which demonstrates that an intracellular gradient of osmotic pressure is capable of transferring water through a cell, at constant temperature, against an external gradient of osmotic pressure. The balance of hydrostatic and osmotic forces developed at the steady state of operation of such a cell is considered.

2. Evidence, which is indicative of the actual existence of intracellular gradients of osmotic pressure and chemical activity in living cells, is adduced. It appears that such gradients, if they exist, may be maintained by the operation of the ordinary metabolic processes of glycolysis and oxidative recovery.

3. Gradients of chemical activity can, in theory, account for selective absorption or excretion of dissolved substances.

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THE MOUNTAIN LAKE BIOLOGICAL STATION OF THE UNIVERSITY OF VIRGINIA[1]

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This paper presented a general discussion of the development of the Mountain Lake Biological Station of the University of Virginia, located in the mountains of southwestern Virginia. The first work at the station was given in the summer of 1930. A grant of $25,000 by the General Education Board made possible the erection of new buildings and the first session at the present location was held in 1934. It is the purpose of the station to serve the Southern States. Eight students from the state of Tennessee have attended the sessions of 1934 and 1935 and a member of the faculty of the University of Tennessee has served upon the teaching staff. One of the cottages at the Mountain Lake Station is to be named for Gattinger, the great Tennessee botanist. The speaker has held a General Education Board Scholarship at the station during the summers of 1934 and 1935. Dr. Ivey F. Lewis, Miller Professor of Biology of the University of Virginia, is the Director of the station and it is through his courtesy that the slides are presented.

[1]Abstract of a paper presented before the Tennessee Academy of Science at the Nashville meeting, November 29, 1935.