SOME ASPECTS OF SECRETION

I. SECRETION OF WATER

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Some problems in the secretion of liquid might be solved if it could be shown that a secreting cell can take up water at one spot on its surface and expel it at another spot.

It is therefore of interest to find that a *Nitella* cell can do this when the internal osmotic pressure is not uniform throughout the cell. This can be shown in a variety of ways.

The procedure is simple. We employ a cell \(^1\) about 8 cm. long; this has a cellulose wall within which is a layer of protoplasm surrounding a large central vacuole. We place it in the apparatus shown in Fig. 1. The cell is surrounded in the center by a soft rubber pencil eraser, \(E\), split lengthwise by a sharp razor after which a groove is cut in one half to receive the cell. In \(E\) the cell is surrounded by a layer of vaseline which makes a water-tight seal so that no water can pass through \(E\). A tube, \(L\), filled with water, is now slipped over the left end of the rubber \(E\) for a distance of about 6 mm. and then withdrawn a little to the left so as to cause air to enter the calibrated capillary for a distance of 4 or 5 cm. so that a meniscus appears in the capillary. A tube, \(R\), filled with water, is now slipped over the right end of \(E\). This tube also ends in a calibrated capillary and a meniscus appears at the tip. The meniscus in each tube remains stationary as long as water remains in \(L\) and \(R\) (these tubes are in a horizontal position).

For convenience we designate the portion of the cell in the tube \(L\) as \(A\) and the portion in the tube \(R\) as \(B\) (Fig. 1).

\(^1\) The observations were made on *Nitella flexilis*, Ag. The cells were freed from neighboring cells and kept in the laboratory in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, 17, 87) at 15°C. ±1°C. About an hour before use the temperature was raised to about 25°C.

\(^2\) The sizes of the rubber eraser \(E\) and of the glass tubes \(L\) and \(R\) are adjusted so that the rubber is firmly pressed around the cell without injuring it. When the seal at \(E\) is properly formed the apparatus can be tilted at an angle to the horizontal without causing a movement of the meniscus.

In order to avoid air bubbles in the tubes \(L\) and \(R\) the entire apparatus may be submerged in water when the tubes are slipped over \(E\) but this is not necessary if care is taken to have the tubes completely filled with water.
We now replace the tube $L$ by a similar tube $L$ filled with 0.24 M sucrose\textsuperscript{4} and admit air into the capillary as before. The meniscus in $R$ and the meniscus in $L$ at once move to the left, showing that the amount of water in $L$ is increasing and the amount in $R$ is decreasing. Since, as shown later on (p. 445), the dimensions of the cell do not change it is evident that water is entering the cell at $B$ and moving out of the cell at $A$. Each meniscus moves several centimeters. After a few minutes the motion stops or becomes too slow to be detected.

The liquid which moves from $B$ to $A$ carries solutes which remain in $A$ as the water leaves the cell since they are unable to pass out through the protoplasm. In consequence the internal osmotic pressure at $A$ increases.

We now replace the tube $L$ by a similar tube $L$ filled with water. At once the meniscus in $L$ moves to the right and the meniscus in $R$ does likewise, showing that water is entering the cell at $A$ and coming out at $B$. Each meniscus moves several centimeters before stopping.

In this way the water can be made to move back and forth several times and the cell if immersed in water can survive 48 hours or more. The cell then shows normal turgor and protoplasmic motion.

\textbf{Fig. 1. A Nitella cell, $AB$, is surrounded in the center by a soft rubber pencil eraser, $E$, to which are attached the tubes $L$ and $R$ which end in calibrated capillaries. By observing the menisci in these we can measure the change in volume of liquid in these tubes. With 0.24 M sucrose in $L$ and water in $R$ we see that water enters the Nitella cell at $B$ and comes out of the cell at $A$. We now replace the sucrose solution at $A$ by water and see that the cell takes up water at $A$ and expels it at $B$.}

In this movement of water the living protoplasm plays an active part since it is necessary to maintain the osmotic properties of the cell. We find practically no movement when we employ a dead cell or a piece of wet string. (Experiments described later on show that the water moves in the protoplasm as well as in the vacuole (p. 445).)

It is therefore evident that a Nitella cell can take up water at one spot on its surface and expel it at another spot when both spots are in contact with identical solutions provided the internal osmotic pressure is not the same at both spots.

What causes these movements of water in the cell? The driving force which

\textsuperscript{4} Preliminary experiments indicate that what is said of sucrose in this paper applies to other sugars and likewise to NaCl of corresponding osmotic pressure. This and other changes are performed as quickly as possible to avoid evaporation from the surface of the cell. The apparatus may be submerged in solution when tubes filled with solution are slipped over $E$.

\textsuperscript{4} This may be clearly seen when the vacuole contains brilliant cresyl blue (cf. Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1946–47, 30, 229). Tests for the exit of Cl$^-$ at $A$ give negative results unless the cell is injured.
makes water enter is assumed to be equal to the excess of internal osmotic pressure over that of the external solution. The average internal osmotic pressure is about 6 atmospheres at 25°C., as shown by plasmolytic tests with sucrose to which the cell is only slightly permeable. Hence at B where the cell is in contact with water the driving force causing water to enter the cell is about 6 atmospheres. At A the cell is in contact with 0.24 M sucrose which has an osmotic pressure of about 6 atmospheres at 25°C., so that the driving force at A is practically zero. Hence water moves from B to A.

Let us now consider the volume of water which moves from B to A. To measure this we prefer the apparatus shown in Fig. 2. With this we proceed as before but all the measurements are made in the capillary of tube R and are more accurate because we do not have to disconnect the tube L to change solutions. By measuring the length and diameter of the cell we can calculate its volume and by measuring the distance traversed by the meniscus we ascertain the volume of water which has left the tube R and entered the cell. Experiments described later (p. 445) show that there is no increase in the amount of liquid in the cell since the cell does not increase in size. This would be expected

![Fig. 2](image-url) 

FIG. 2. As in Fig. 1 but the tube L ends in a wide orifice instead of a capillary. This makes it possible to change solutions in the tube L without detaching it.

since the cellulose wall is too resistant to permit further expansion except by a very slow process of growth. Evidently as much water as passes into the cell at B passes out at A.

The following calculation indicates that the water moves in each direction under a driving force of about 6 atmospheres when the volumes of A and B are equal.

Let us first consider the motion from B to A. This will continue as long as the inwardly directed driving force is greater at B than at A. At B, where the cell is in contact with water, it amounts to about 6 atmospheres. When we put 0.24 M sucrose at A the driving force becomes zero at A since the osmotic pressure of the sucrose solution is about 6 atmospheres. Hence water moves from B to A, carrying solutes with it.

The passage of solutes from B to A lowers the driving force at B and raises it at A since the solutes do not pass out of the cell at A. As a result the driving forces at A and B become equal and the motion stops because the equal opposing driving forces at A and B tend to force water into the cell and the cell cannot expand.

The amount of driving force which must be subtracted from B and added to

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A to equalize the two driving forces may be called $D_T$; the driving force at $A$
may be called $D_A$ and that at $B$ designated as $D_B$. We may then write

$$D_T = \frac{D_B - D_A}{2} = \frac{6 - 0}{2} = 3 \text{ atmospheres}$$

Hence we must move enough solutes from $B$ to $A$ to take away 3 atmospheres
from $B$ which means that half the liquid in $B$ must move to $A$ (since the internal
osmotic pressure at $B$ is 6 atmospheres).\(^6\) If we assume that the volume of the
cell enclosed in $E$ is so small as to be negligible we may say that half the liquid
in $B$ is 25 per cent of the total liquid in the cell.\(^7\) Hence 25 per cent of the total
liquid in the cell should move from $B$ to $A$ before the motion stops.

In practice the volume of the cell enclosed in $E$ is too large to be neglected
and we consider it as part of $B$ since it is not in contact with sucrose and liquid
passes from it to $A$.

When determinations were made on this basis, 6 observations gave an aver-
age value of 28.7 ± 1.6 per cent for the volume of water moving.

This may indicate that the average value of the internal osmotic pressure of
these cells was a little less than the assumed value of 6 atmospheres.

The internal osmotic pressure varies and consequently the observed values
may diverge from the calculated value of 25 per cent which is based on the
assumption that the internal osmotic pressure is 6 atmospheres (so that $D_A = 0$
and $D_B = 6$). But if the internal osmotic pressure is 4 atmospheres we
have $D_A = -2$ and $D_B = 4$, and the calculation shows that 37.5 per cent
of water should move. If the internal osmotic pressure is 7 atmospheres the calcu-
lated amount of water moving is 21.5 per cent.

In considering the observed values it should be borne in mind that if water
passes from $B$ to $A$ without carrying as much solute as the calculation assumes
the observed percentage will be larger than the calculated. This will also be
the case if normal protoplasmic motion continues and carries solutes back
from $A$ to $B$.

When the volumes of $A$ and $B$ differ a formula may be used which is set up
as follows. Let us suppose that the volume of $A$ is 2 and the driving force at

\(^6\) The internal osmotic pressure at $A$ becomes 9 atmospheres and since the osmotic
pressure of the external sucrose solution is 6 atmospheres there is a driving force of $9
- 6 = 3 \text{ atmospheres at } A$, tending to force water into the cell.

\(^7\) The volume of the cellulose wall is not regarded as part of the volume of the cell.

In these calculations we disregard the dilution of the sucrose by water escaping at
$A$ but this is relatively small and the dilution of the 0.24 m sucrose is evidently not
important since if we put on fresh sucrose solution we see little or no effect.
A (called $D_A$) is 1 atmosphere; the volume of $B$ is 1 and the driving force (called $D_B$) is 10 atmospheres (the volume of $B$ includes the volume enclosed in $E$).

The excess of driving force at $B$ is $10 - 1 = 9$ atmospheres. We now remove from $B$ sufficient solutes to produce 9 atmospheres if they remain in $B$ or 3 atmospheres if distributed uniformly throughout the cell (which has 3 times the volume of $B$). Let this distribution be made. We then have in $A$ the original driving force of 1 atmosphere plus the 3 just added, making 4 in all. We may call this $D_\text{r}$ and write

$$D_\text{r} = \frac{D_B - D_A}{V_{\text{cell}} + V_B} + D_A$$

where $D_A$ is the driving force in $A$ at the start and $D_B$ that in $B$ at the start, $V_{\text{cell}}$ is the volume of the cell, and $V_B$ is the volume of $B$. Substituting values we have

$$D_\text{r} = \frac{10 - 1}{3 + 1} + 1 = 4 \text{ atmospheres}$$

In $B$ we had 10 atmospheres at the start but we removed 9 and then put back 3 (when the solutes were distributed throughout the cell) so that we now have $1 + 3 = 4$ atmospheres, just as in $A$.

The motion now ceases since the driving forces in $A$ and $B$ are equal and each tends to force water into the cell which cannot expand because of the cellulose wall.

Let us now consider the driving force at $B$. At the start it is 10 atmospheres and at the end 4 atmospheres, hence the loss is 6 atmospheres. If the per cent of loss in $B$ is called $L_B$ we may write

$$L_B = \left(\frac{D_B - D_\text{r}}{D_B}\right) \times 100$$

$$= \frac{10 - 4}{10} \times 100$$

$$= 60 \text{ per cent}$$

This means that 60 per cent of the driving force at $B$ is lost. We may assume that the movement involves 60 per cent of the solutes and consequently 60 per cent of the liquid in $B$. Hence we may say that the motion stops when water amounting to 60 per cent of the volume of $B$ has moved. This is 20 per

We assume that the osmotic pressure inside is 10 atmospheres and that of the external sucrose solution is 9 atmospheres.
cent of the volume of the cell (which has 3 times that of \( B \)). If the moving liquid expressed as per cent of the liquid in the cell is called \( L \) we may write

\[
L = L_0 \left( \frac{V_B}{V_{cell}} \right)
\]

According to such calculations the maximum movement of water which can occur (unless \( D_A \) is negative) is 25 per cent of the total volume. This occurs when the volume of \( A \) is half the total volume and \( D_A = 0 \).

Let us now return to the original experiment (p. 440) where the volumes of \( A \) and \( B \) are equal and consider the movement of liquid from \( A \) to \( B \) when the sucrose at \( A \) is replaced by water. Previous to this movement there was a movement from \( B \) to \( A \) producing 9 atmospheres internal osmotic pressure at \( A \) and 3 atmospheres at \( B \); hence when \( A \) and \( B \) are both in contact with water we have a net driving force of \( 9 - 3 = 6 \) atmospheres causing water to move from \( A \) to \( B \).

If we use the apparatus shown in Fig. 2 we find that when sucrose at \( A \) is replaced by water there is a movement of liquid from \( A \) to \( B \) and as a rule the volume is approximately equal to that which has previously moved from \( B \) to \( A \). If this is not the case the experiment is rejected (this may happen with the later tests when a long series of tests is made on the same cell).

A model to imitate the osmotic behavior of \textit{Nitella} might be constructed by using 2 porous pots made impermeable to sucrose by impregnation with copper ferrocyanide, such as are used in measurements of osmotic pressure. By filling these with 0.24 M sucrose, connecting them by a glass tube, and then immersing one \((A)\) in 0.24 M sucrose and the other \((B)\) in water we should have a model behaving osmotically like the \textit{Nitella} cell. Water would enter \( B \) and move to \( A \) carrying sucrose with it. The internal concentration of sucrose in \( A \) would increase since water would pass out at \( A \) but the sucrose would be left behind as it could not pass out through the ferrocyanide membrane. Hence the internal concentration of sucrose at \( A \) would increase and if water were substituted for the external solution of sucrose water would enter at \( A \) and come out at \( B \) since the driving force would be greater at \( A \) than at \( B \).

Let us now consider some qualitative observations made with simpler technique.

We place a cell about 8 cm. long on a microscope slide without a coverglass. A region about 6 mm. long in the center of the cell is covered with vaseline which surrounds the cell and adheres to the slide. This effectively prevents any mixing of different solutions applied simultaneously to opposite ends of the cell. For convenience we designate the entire region to the left of the vaseline as \( A \) and the region to the right of the vaseline as \( B \). The cell is observed under the microscope.

With water at \( A \) and \( B \) the protoplasm and vacuole show only a slow move-
ment of particles. If we now replace the water at A by 0.24 M sucrose, keeping water at B, we see a very striking change. All the particles in the vacuole rush rapidly to A and remain there as long as the sucrose remains at A and water remains at B. In many cases the motion of particles in the protoplasm agrees with that in the vacuole in that all the particles move from B to A.9

The rush of particles in the cell soon stops. This is to be expected for reasons already stated (p. 441). When this happens we replace the sucrose solution at A by water, while water remains at B. This produces an immediate rush10 of particles in the vacuole from A to B, which may be accompanied by a similar rush in the protoplasm. This is likewise to be expected for reasons already stated (p. 441).

This means that water enters at A, travels to B, and escapes at B for careful measurements show that the volume of the cell does not change (this is due to the resistant cellulose wall which cannot expand except by a very slow process of growth).

To show that the exit of water from B may take place when the whole cell is covered with water we vary the experiment as follows. In place of a vaseline barrier we use a barrier of gelatin11 and after the internal osmotic pressure at A has been raised by the application of sucrose solution we remove the gelatin barrier and submerge the entire cell in water. We then see a rapid rush of particles from A to B.12 When the cell has been stained with brilliant cresyl blue as described in a previous paper1 the motion of dye in the vacuole agrees in direction with that of the particles.13 This means that water enters at A and comes out at B.

It is therefore evident that if a secreting cell has a relatively high internal osmotic pressure in any region it may take up water in this region and expel it in another region although the entire cell is in contact with water.

These experiments may serve to visualize the situation in a secreting cell which is in contact with liquid over its entire surface. In some plants water appears to be given out at a spot where the cell is in contact with air. In view

9 In other cases, especially when the rush is less violent, the particles in the protoplasm may continue to move just as they do under normal conditions; i.e., up one side of the cell and down the other.

On each side of the cell a “white line” (a narrow band containing no chloroplasts) extends the entire length of the cell. In these two lines there is no normal protoplasmic motion and water might therefore go in and out in these lines without disturbing the normal protoplasmic motion.

10 As stated in a previous paper (Osterhout, W. J. V., J. Gen. Physiol., 1946-47, 30, 229) the chloroplasts contract in the ingoing current and expand again when the current is reversed.

11 In place of vaseline we may use 20 per cent gelatin warmed to 29°C to make it fluid. This soon stiffens on cooling and does not injure the cell.

of this the following experiment was made which shows that water can come out where the cell is in contact with air.

A cell was placed on a slide on the stage of a microscope and covered with water at B; the water extended to a vaseline barrier in the middle of the cell which effectively prevented the water at B from mixing with 0.24 M sucrose placed at A. The sucrose at A caused a rush of liquid in the vacuole and in the protoplasm from B to A. Then the cell at B was carefully dried with filter paper. At B the cell was held a little above the slide and did not come in contact with it (this was done by appropriate adjustment of the vaseline barrier). As an additional precaution against movement of external liquid from A to B the stage of the microscope was tilted so that B was higher than A. When 0.24 M sucrose at A was replaced by water there was a rush of liquid in the protoplasm and vacuole from A to B, and drops of water came out of the cell into the air at B.

In some cases the portion of the secreting cell at which water is expelled may be in contact with the non-aqueous surface layers of other cells. In view of this a cell was arranged in the manner just described but it remained horizontal and in place of air at B the cell was in contact with light paraffin oil (for medicinal use). In this case drops of water appeared in the oil just outside the cell.

DISCUSSION

It would seem that if suitable inequalities in osmotic pressure exist in the cell it can take up water at one place and expel it at another. It is evident that if such cells were suitably arranged in a gland or in a tissue they might bring about the secretion of water. The question arises whether such inequalities may occur as the result of local inequalities in metabolism in different regions of a cell.

The metabolism at the outer surfaces of the superficial cells of the root may be different from that at their inner surfaces since at the outer surfaces there is a more abundant supply of mineral nutrients and of oxygen than at the inner surface. This is especially true of the cells which produce root hairs and which play the principal rôle in absorption of water wherever they occur. A root hair is merely an outgrowth from one of the superficial cells of the root and usually contains the nucleus. It is a long cylindrical structure which might be compared to a Nitella cell in its proportions. It would not be surprising if the metabolism of that part of the cell which grows out as a long cylinder into the external solution created a higher osmotic pressure than in the basal por-

14 This operation was performed as expeditiously as possible in order to avoid evaporation at B.

15 When the cell was covered with water soon afterwards it showed no signs of injury.
tion of the cell which is surrounded by other cells. By analogy with the Nitella cell we might expect water to be expelled under pressure from the basal part of the cell and hence to contribute to the pressure which causes the sap to move upward (root pressure).

In animal tissues which secrete water there would seem to be every opportunity for differences in metabolism to arise in different regions of the cell since the tissue may obtain water from the blood on one side and discharge it into the lumen of the gland on the other. To bring about secretion there must be some mechanical restriction which prevents the cell from expanding indefinitely as water is absorbed. That such a restriction exists is evident from the mechanical properties of the gland tissue. It may be that when the secretion in a gland occurs as the result of nervous stimulation each secreting cell is stimulated more strongly on one side with a consequent increase in metabolism and in internal osmotic pressure on that side.

In such tissues it does not seem probable that the secreted water is derived from the death of cells. This process might provide proteins and other solutes but would seem hardly adequate for the secretion of water.

It seems justifiable to conclude that suitable inequalities of osmotic pressure occurring periodically in secreting cells might bring about the periodic secretion of water. To what extent this occurs in any individual case must be determined by investigation.

Measurements of permeability to water are being made by means of the apparatus described here and may be presented later.

**SUMMARY**

If we increase the osmotic pressure at one end of a Nitella cell by applying a solution of sucrose and if we subsequently submerge the entire cell in water we find that water enters at the end where the osmotic pressure is higher and comes out of the cell at the other end.

If similar inequalities of osmotic pressure should arise as the result of metabolism we can understand how a secreting cell might take up water at one spot on its surface and expel it in another spot and thus bring about the secretion of water.

The Nitella cell can expel water from a region of the cell which is in contact with water, air, or mineral oil.