THE PHYSICAL STATE OF WATER IN FROG MUSCLES

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SUMMARY

By using a vapor-equilibration method, the equilibrium water content of frog voluntary muscles was studied over the range of relative vapor pressures from 0.906 ("isotonic") to 0.043. Comparing the data with that obtained by the standard immersion method, we found that at relative vapor pressure between 0.986 and 0.996, the two methods yield similar data. A plot of water content against osmotic pressure yields a straight line only at vapor pressures above 0.985 ($1/\pi = 0.0495$). The entire set of data can be described by a Bradley multilayer isotherm superimposed upon a monomolecular adsorption isotherm, with the multilayer fraction making up about 95% of the resting muscle water content and the smaller, more tightly adsorbed fraction making up the remaining 5%. This separation of two fractions of polarized water agrees with similar conclusions from three other sets of independent investigations. These data are shown to be consistent with the association-induction hypothesis but not with the membrane theory. The data add indirect evidence that the bulk of intracellular K^+ ion and organic anions is in an absorbed state.

There is increasing evidence, from both nuclear magnetic resonance studies and intracellular freezing experiments, that the water within living cells is in a different physical state than water in a dilute salt solution.[1-6] These findings are in harmony with the theory that the bulk of intracellular water exists as polarized multilayers on cellular proteins.[7] The theory also predicts a hyperbolic relation between solute concentration in the medium and cell volume[8]—a relation often considered as unique evidence in support of the membrane theory.[9,10]

In this paper we present results of our studies on the change of muscle cell volume in response to variation of the water activity in the cell's environment. The method used does not involve direct contact between the tissue and the aqueous solutions providing different water activities. We have therefore been able to explore the entire range of water activities, a task difficult to achieve using the conventional soaking method. Our method also eliminates experimental errors due to changes in the intracellular solute contents which usually accompany soaking (see below).

MATERIALS AND METHODS

Equilibrium in Vapor Phase

The method basically consists of equilibrating sterilely dissected small muscle strips from the sartorius muscle in hermetically sealed vessels containing water vapor at different vapor pressures provided by different NaCl and H_2SO_4 solutions (see ref. 11).

Data on the concentration of H_2SO_4 and NaCl providing known vapor pressures at 25°C were obtained from International Critical Tables[12] and from Frazer.[13] NaCl solutions were used to obtain relative vapor pressures of 0.995 and above: H_2SO_4 solutions for those lower than 0.995.

Pyrex tubes (10 x 75 mm) with pleated 5 x 6 cm filter paper inserted, pippettes, etc. were sterilized by autoclaving and were then dried at 105°C. Size O rubber stoppers with a loop of glass thread sewn to the bottom were sterilized by soaking in 70% alcohol for 2 hours, and then dried under germicidal ultraviolet light for 1 hour. Three cc of H_2SO_4 or NaCl solution were pipetted into each tube, after which the tube was immediately closed with a tight-fitting rubber stopper. This assembly was allowed to stand for 24 hours in a constant temperature bath slightly below 25°C.

Leopard frogs (Rana *pipiens*, Schreiber) were kept in running water at room temperature and fed hamburger meat regularly. After the frog was rinsed in tap water, its skull was crushed with rongeurs and the skin incised completely around the thorax below the forelegs and stripped down. The spinal cord was crushed at about 3 mm above the pelvic joint. The sartorius muscles were then either isolated under sterile conditions in a straightforward manner and studied in their intact state. or they were isolated from their surrounding tissues except at the point of origin. With the aid of fine forceps a muscle was split lengthwise into several strips and severed from the pelvic origin by careful dissection to minimize the number of muscle fibers injured. Each strip was threaded through and thus suspended on the glass thread loop on a rubber stopper. The stopper with the muscle strip was then quickly substituted for the plain stopper on a pre-equilibrated tube. The tube was sealed with paraffin film and totally immersed in an Aminco constant temperature water bath at 25°C. To prevent condensation, the room temperature was maintained at a degree or so below 25°C during operations leading to the immersion of the tubes. The bath temperature was kept at 25°C ± 0.05°C (larger fluctuations would lead to vapor condensation at higher vapor pressures).

Water Content

Water content, expressed as g H_2O/100 g dry muscle, was determined from the difference between the wet weight measured on a torsion balance immediately after removal of the muscle strip from the equilibration tube, and the dry weight measured after drying in an aluminum pan at room temperature for several hours and then in an oven at 105°C for

24 hours.

Equilibration in Sucrose Ringer Solutions

Paired muscles were isolated as described above. One was then immersed in 100 ml of solution while the water content of the other was assayed immediately. The composition of the solution was KCl 2.5 mM, $NaHCO_3$ 6.0 mM, NaH_2PO_4 2.0 mM, Na_2HPO_4 1.2 mM, $CaCl_2$ 1.0 mM, $MgSO_4$ 1.2 mM, sucrose variable (0.05 to 6.0 M). Vapor pressures were estimated using data from Frazer[13] assuming the vapor pressure of the salts in the solution to be equivalent to 0.014 M NaCl, or 0.035 M sucrose, (relative vapor pressure 0.995). Water contents of muscles in these experiments were calculated according to the following formula:

$$\frac{g\,H_2O}{100\,g\text{ dry muscle}} = \frac{100\,[Wf_2 - Ws - (Wd_1/Wi_1) \times Wi_2]}{(Wd_1/Wi_1) \times Wi_2} \qquad (1)$$

where Wi_1 was the initial wet weight and Wd_1 the dry weight of the paired control muscle: Wi_2 was the initial wet weight and Wf_2 the final wet weight of the experimental muscle: and Ws was the weight of sucrose in the experimental muscle, obtained from the difference between actual dry weight and that expected from the control muscle.

RESULTS

Time Course and Reversibility

The water content of frog sartorius muscles immersed in hypotonic sucrose Ringer solutions reached equilibrium in about 3 hours: In hypertonic solutions it took 50 min to reach a plateau, but there was a secondary gain in weight, suggesting a slow entry of sucrose into cells (Fig. 1). The water contents of muscles exposed to varying vapor pressures (25°C) reached equilibrium by 100 hours and is maintained for at least another 100 hours (Fig. 2).

To test the reversibility of the steady level water contents (Fig. 3, Table 1), muscle strips were first brought to equilibrium at a low vapor pressure and then rapidly transferred to a tube containing a higher vapor pressure (p/p° = 0.9954 or 0.9960). Complete reversal to about 300 g H_2O/100 g dry muscle (75% water) at p/p° = 0.9954, was achieved in muscles with equilibrium water contents above 200 g H_2O/100 g dry muscle. Muscles whose equilibrium water content had fallen below this value also regained water, but final value reached was lower.

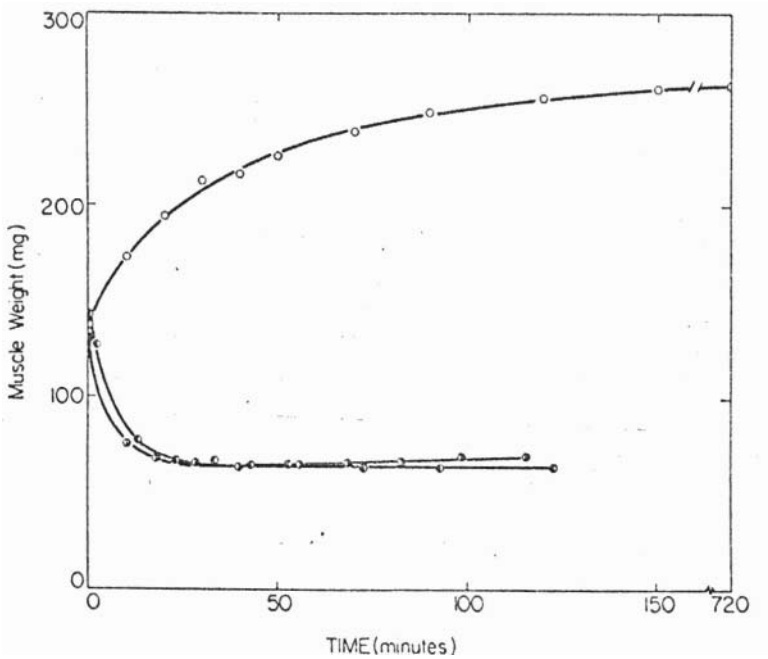

Figure 1. Time course of equilibrium watcr content in frog sartorius muscles immersed in sucrose Ringer solutions at 25°C.
○, p/p° = 0.9987; ◑, p/p^{O} = 0.905 (4.0 M sucrosc); ◐, p/p^{O} = 0.860 (5.0 M sucrosc). Each time courx represents one muscle.

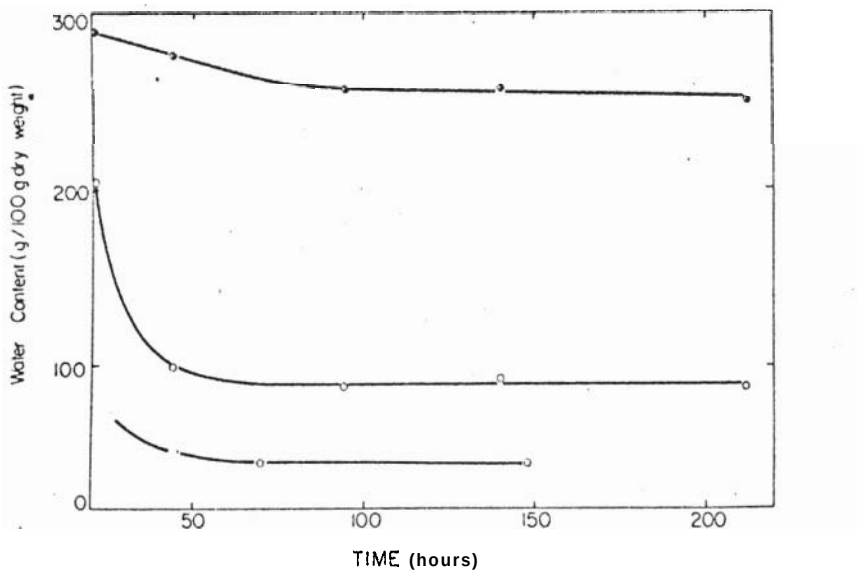

Figure 2. Time course of cquilibrium watcr content in frog sartorius muscles cxposcd to watcr vapor at 25°C.
Each point is the average of 4 muscle strips for the upper and middle curves: it is the value of a single muscle strip for the lower curve. Lower curve, p/p^{O} = 0.909; middle curve, p/p° = 0.953; upper curve, p/p^{O} = 0.993.

Table 1. Reversal of water content after equilibrium at lower vapor pressures.

Time courses for reversal at p/p^o 0.9954 are shown in Figure 3; only final equilibrium water contents of reversal are given here. When approached from isotonicity (p/p^o 0.900) (350-400 g dry weight) the equilibrium water content at p/p^o 0.9954 is about 300 g H_2O/100 g dry weight. Number in brackets refers to the number of experiments performed.

p/p^o	Initial Water Content (gm./100 gm. dry weight)	p/p^o	Final (Reversal) Water Content (gm./100 gm. dry weight)
0.909	75.3 ± 5.9(3)	0.9954	195 ± 5.6(5)
	50.4 ± 1.8(5)		195 ± 5.9(3)
	45.1 ± 1.4(4)		187 ± 6.5(4)
0.970	273 ± 8.0(4)	0.9954	192 ± 18.4(4)
	131 ± 8.0(4)		219 ± 18.0(6)
	109 ± 8.0(4)		218 ± 8.0(4)
0.981	159 ± 13 (4)	0.9954	259 ± 10.2(6)
		0.9960	311 + 21.3(4)
0.990	221 ± 3.1(3)	0.9954	292 ± 16.5(5)
		0.9960	213 ± 6.4(7)
0.993	(250)	0.9954	310 ± 10.0(4)
		0.9960	291 ± 17.1(6)

Although muscles exposed to a vapor pressure lower than that of normal Ringer solution (p/p^o = 0.9969) lost similar amounts of water as those soaked in a hypertonic solution (see below), niusclcs exposed to vapor pressures higher than this value gained less weight or actually lost some weight (Table 2). However, when these muscles were subsequently immersed in a hypotonic Ringer solution they readily swelled to final weights approaching those of freshly isolated muscle in a similar hypotonic solution (bracketed figures in Table 2).

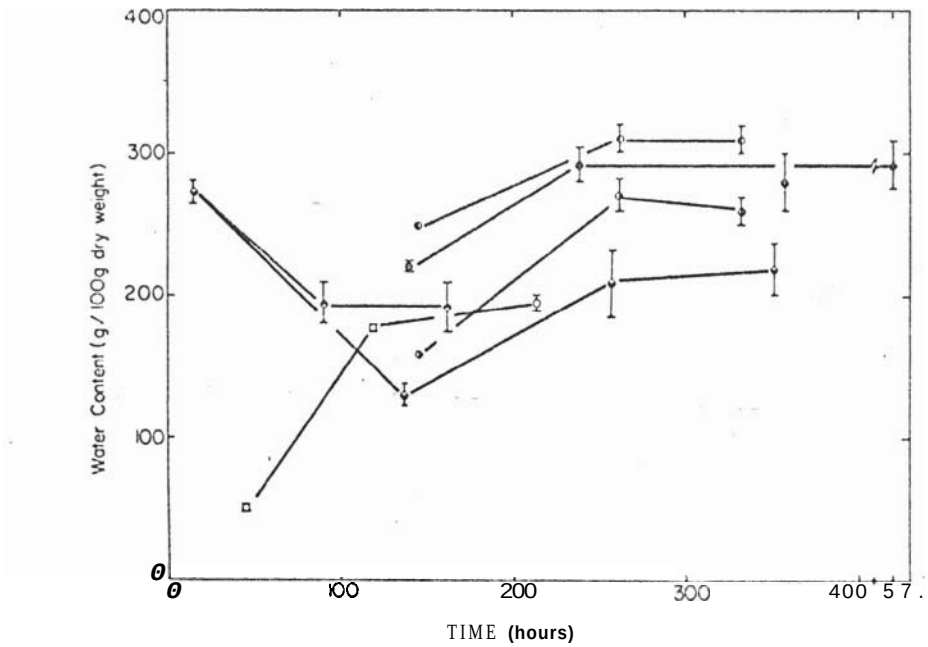

Figure 3. Reversal of cquilibriuni water contents of frog sartorius muscles csposcd to watcr vapor at 25°C.
Data summarized in Table 1; shown hcrc, reversal at p/p° = 0.9954 from the following: ○, p/p° = 0.909: ◐, p/p° = 0.970; ◒, p/p° = 0.979; ◑, p/p° = 0.981: ●, p/p° = 0.990; ◓, p/p° = 0.993. Computed SE (n = 4-6).

The Water Content of Muscle Strips *in Equilibrium with Different* Vapor Pressures

The watcr content of muscle strips in equilibrium with watcr prcssurcs from p/p° = 0.033 to p/p° = 0.096 is shown in Table 3 (for display of experimental points see Fig. 9). The overall shape of the isotherm resembles that seen, in carlicr studics, in gaseous adsorption on solid surfaces[14,15] and in watcr adsorption on proteins.[16] It differs from that sccn in these carlicr studies in that the adsorption isotherm has been cxtcndcd to much higher relative vapor prcssurcs.

That the high water content measured at these very high vapor prcssurcs was not due to 3 systematic error (e.g., capillary condensation) is shown by comparing the water contents so measured (half-filled circles in Fig. 4) with those obtained by the conventional method of immersing muscles in Ringer sucrose solution (empty Fig. 4). Above a rclative vapor pressure of 0.986 the data obtained by the vapor method and by the soaking method fall on the same line.

Below the relative vapor pressure 'of 0.986 the two sets of data no longer agree. The data obtained by the conventional method now lie above those obtained by the vapor

Table 2. Equilibrium water content of muscles at relative vapor pressures above isotonicity and after subsequent immersion in hypotonic Ringer solution.
Isotonic relative vapor pressure is $p/p^o = 0.9960$. Number in parentheses refers to the number of experiments. Muscle strips that had been exposed to pure water vapor ($p/p^o = 1.000$) gained some weight (water content of normal muscle in isotonic solution is from 400 to 430 g per 100 g of dry weight); They gained more water when subsequently the muscle strips were soaked for 30 minutes in a hypotonic Ringer solution, containing only 30% of the normal amount of NaCl in a Ringer solution. The final weights achieved approached those of freshly isolated muscle strips similarly treated (bracketed figures).

After Vapor Equilibrium		Subsequent Equilibration in Solution	
p/p^o	Water Content (gm./100 gm. dry weight)	p/p^o	Water Content (gm./100 gm. dry weight)
1.0000	572 ± 31 (4)	0.999	802 ± 35 (4)
0.9903	409 ± 10 (5)	[0.999	047 + 22 (6)]
0.9987	361 ± 12 (4)		
0.9974	370 ± 44 (3)		
0.9960	372 ± 27 (12)		

phase equilibrium method. It is noteworthy that this relative vapor pressure of 0.986 also marks the point beyond which the muscles no longer effectively exclude the sucrose in the soaking medium. Thus Table 4 and Figure 5 show that at a sucrose concentration below 0.7 molal (relative vapor pressure above 0.986), the sucrose content of the muscles remain in the neighborhood of 22% of that in the surrounding medium (see also 17). As the sucrose concentration of the surrounding solution becomes higher than 0.7 molal, the sucrose content of the muscle abruptly rises to a higher level. Thus the higher water content in the more concentrated sucrose solution can be ascribed to the additional osmotic activity of the extra amount of intracellular sucrose and other changes accompanying this large sucrose entry.

Table 3. The equilibrium water contents of frog sartorius muscle strips at 25°C. Equilibrium time for all experiments was between 7 to 8 days.

Relative Vapor Pressure (p/p$_s$)	Number of Experiments	(gm. H_2O/100 gms. dry wt.) mean ± SE
0.043	4	2.0 ± 0.2
0.356	4	6.5 ± 0.1
0.568	7	12 ± 1.3
0.749	3	14 ± 1.5
0.876	4	31 ± 1.5
0.909	4	45 ± 1.4
0.927	3	50 ± 1.5
0.940	3	61 ± 1.2
0.953	•	88
0.970	3	121 ± 2.9
0.981	4	159 ± 13
0.985	3	196 ± 12
0.990	4	209 ± 14.2
0.993	•	250
0.994	4	283 ± 17
0.995	45	307 ± 14.6
0.996	12	372 ± 2.7

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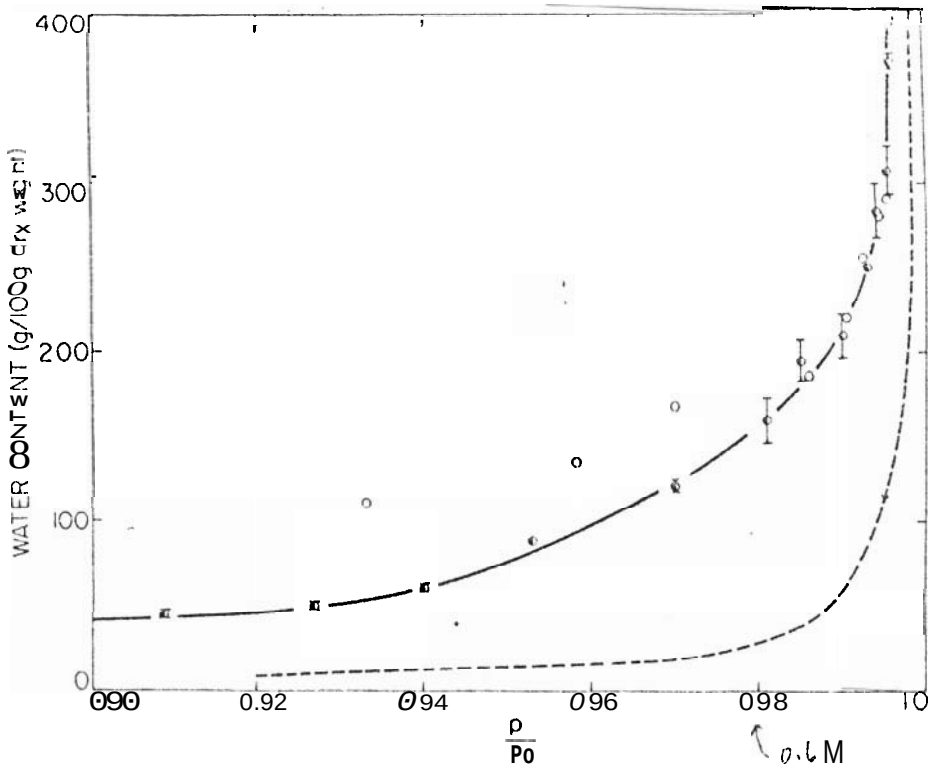

Figure 4. Comparison of equilibrium water content of frog sartorius muscles in sucrose Ringer solution with that exposed to water vapor. 25°C.
●, water vapor data from Table 3: ○, sucrose Ringer data from Table 4 calculated by Equation 12. Dotted line represents weight of water associated with 100 g of NaCl at a specified relative vapor pressure (data from 13).

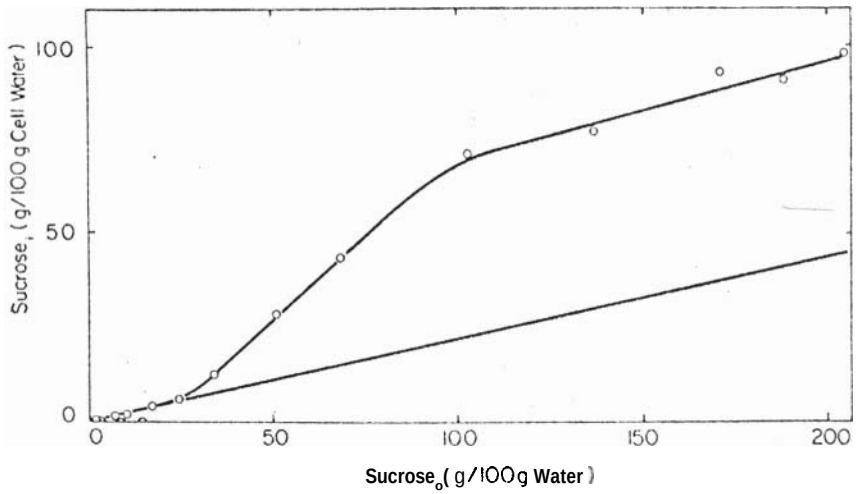

Figure 5. Equilibrium distribution of sucrose in the cell water in sucrose Ringer solutions, 25°C. Data are calculated from those of Table 3. The straight line is at a constant ratio of internal to external sucrose concentration of 0.22. The last point on this line is at p/p° 0.986 (0.7 M sucrose). The ordinate represents the equilibrium concentration of sucrose in 100 g of cell water; the abscissa, that in 100 g of water in the bathing solution.

Table 4. Equilibrium water content of muscles in sucrosc Ringer solutions.
Column 1 represents the concentration of sucrosc in the sucrosc Ringer solution in two units: molality and grams per 100 g H_2O. Wi_1 is initial and Wd_1 is dry weight of the paired control muscle; Wi_2 is initial, Wf_2 is final (after equilibration), and Wd_2 is dry weight of the experimental musclc, Ws, wcight of sucrosc, is $Wd - (Wd_1/Wi_1)Wi_2$. Water content is calculated by Equation 12.

$[Sucrose]_{ex}$		p/p°	$(Wd_1/Wi_1)Wi_2$	Wf_2	Wd_2	Ws	Water Content
Molal Conc.	g /100g. H_2O		mg.	mg.	mg.	mg.	gm./100 gm. dry weight
6.0	206	0.840	34.8	76.2	55.3	20.5	60.7
5.5	188	0.860	41.8	102.0	70.5	28.7	75.4
5.0	171	0.876	42.5	109.7	74.9	32.4	81.9
4.0	137	0.905	42.7	113.0	73.4	30.7	92.8
3.0	103	0.933	28.4	82.6	51.0	31.6	111
2.0	685	0.958	40.1	119.0	64.0	23.9	137
1.5	51.4	0.970	34.0	107.4	50.5	16.5	167
1.0	34.2	0.980	47.7	139.2	58.0	10.3	170
0.7	24.0	0.986	26.5	78.4	29.4	2.9	185
0.5	17.1	0.9903	36.3	119.6	39.5	3.2	221
0.4	13.7	0.9924	40.5	140.2	39.4	(0)	255
0.3	10.3	0.9943	36.0	139.0	37.8	1.8	281
0.25	8.55	0.9953	31.2	113.0	29.0	(0)	290
0.2	6.85	0.9961	38.4	191.0	40.7	2.3	394
0.1	3.42	0.9978	45.9	316.0	45.4	(0)	595
0.05	1.71	0.9987	33.2	316.0	34.7	1.5	847

DISCUSSION

The Membrane Tlzeory

The mcmbranc theory owes its current popularity to two sets of experimental findings concerning the equilibrium distribution of water in living cells: (a) A dcmonstratcd vapor equilibrium with a solution containing free Na^+ and Cl^- ion st a concentration (0.118 M) roughly equivalent to that assayed for the intracellular ions (K^+, crcatine phosphate, ATP, etc.)[18-20] and (b) a repeatedly confirmed hyperbolic relation between "impermeant"

solute concentration in the bathing medium and the cell volume.[21-31] For a long time no alternative theory could account for these facts. Let us now discuss these findings separately.

(1) Isotonicity with 0.118 M NaCl lends strong support to the membrane theory, as long as one can sustain the underlying assumption that the bulk of intracellular water is not materially altered by the large quantity of proteins present in the cell. In the past few years, there has been rapidly gathering evidence that the bulk of cell water is under the polarizing influence of the proteins.[1-6] Isotonicity with 0.118 M NaCl is therefore no longer a support for the membrane theory: indeed, it suggests that a major portion of the K^+ ion and other solutes must be in an adsorbed state, or in some otherwise osmotically inactive state. Were this not the case, the cell should be in vapor equilibrium with a NaCl solution higher than 0.118 M in concentration.

(2) The membrane theory predicts a hyperbolic relation between equilibrium cell volume and the concentrations of what historically have been referred to as impermeant solutes, notably Na^+ ion and sucrose. Again, this is valid only as long as these solutes are absolutely or effectively impermeant. Neither of these conditions are fulfilled by Na^+ ion (refs. 32, 33), or by sucrose (ref. 17, see also Table 4 and Fig. 5).

The Problem of Extracellular Space

Before analyzing the data quantitatively we want to mention that, historically, correction for the water in the extracellular space has been a standard procedure. The volume attributed to the extracellular space may be as large as 20 to 30% of the total muscle. However, investigations of volume changes of isolated single muscle fibers (which have no extracellular space) have not yielded data sharply different from the data from whole sartorius muscle studies (see ref. 27, for example).

Recently, we have undertaken an extensive investigation of the volume of the extracellular space in frog muscles using 3 new methods: equilibrium distribution of poly-L-glutamate,[34] total free Na^+ ion assayed with nuclear magnetic resonance spectroscopy,[35] and demonstration of entry of D-mannitol and sucrose into single isolated muscle fibers mentioned above.[17] All consistently show that the extracellular space proper of frog sartorius muscle cannot exceed 10% and is probably closer to 5%. This revision of extracellular space volume reduces the need for its correction. There is, however, an additional reason to make such correction unnecessary for the present study.

The extracellular space is, in essence, space filled with a 0.1 M NaCl solution. The dotted line in Figure 4 shows the equilibrium weight of water associated with 100 g of dry NaCl at different relative vapor pressures.[13] With lowering of vapor pressure the NaCl solution loses water much more precipitously than the muscle tissue does. Each liter of a 0.1 M NaCl contains about 6 grams of dry NaCl. Assuming that the extracellular space is 10% of the fresh muscle weight, we find that there are about 0.6 g of NaCl in 1000 grams

of fresh muscle which contains altogether 300 grams of dry matter. Thus, for each 100 g of total dry matter, there are 0.3 g of dry NaCl in the extracellular space.

At the relative vapor pressure, say, of 0.995, the water associated with 100 g of dry NaCl is from the dotted curve of Figure 4, about 115 grams. The amount of water associated with 0.3 gram of dry NaCl is therefore $115 \times \frac{0.3}{100} = 0.345$ g. At this vapor pressure, the total water content associated with 100 g of dry muscle is 200 grams. The water associated with NaCl in the extracellular space is therefore $\frac{0.345}{290} = 0.12\%$ of the total content. This is far below our experimental error. At vapor pressure below 0.995, the error introduced is even smaller. Since 0.996 is the upper limit of the range of this study, we conclude that for the present study we can safely ignore the extracellular space.

The van't Hoff Equation

According to the membrane theory, the water content of living cells should follow the van't Hoff equation:

$$\pi \bar{V} = nRT, \tag{2}$$

where R and T are the gas constant and absolute temperature respectively, π is the osmotic pressure, and $\bar{V}'$ is the volume of water associated with n moles of solute in the system.

Using the conventional soaking method, the range of π investigated is very limited. In the present study, the air space separating the cell and the H_2SO_4-H_2O mixtures is ideally semipermeable. It effectively eliminates undesirable and harmful movements of solutes into and out of the cells, yet permits the movement of water. Under these conditions the amount of solutes in the cell, n, remains constant. Equation 2 can be written as:

$$\bar{V}' = \frac{\text{Constant}}{\pi} \tag{3}$$

In Figure 6, the experimental data are plotted as a function of osmotic pressure, π, derived from the relative vapor pressure p/p° and the partial molar volumes ($\bar{V}'$) of water in sulfuric acid-water mixture by the relation:

$$\pi = \frac{RT}{\bar{V}'} \ln \frac{p^\circ}{p}. \tag{4}$$

$\bar{V}'$ was calculated from tlic density data[12] by the following equation (ref. 36, p. 670):

$$\bar{V}' = M_i/1000\,[\rho - c(d\rho/dc)], \tag{5}$$

where M_i is molecular weight of solvent, p is the density of tlic solution of concentrations c (g/g). Equation 3 predicts that a plot $\frac{1}{\pi}$ against tlic water content of the cell, $\bar{V}'$, should yield a simple straight line.

Figure 6 shows that this is true in the range of watcr activity usually investigated by

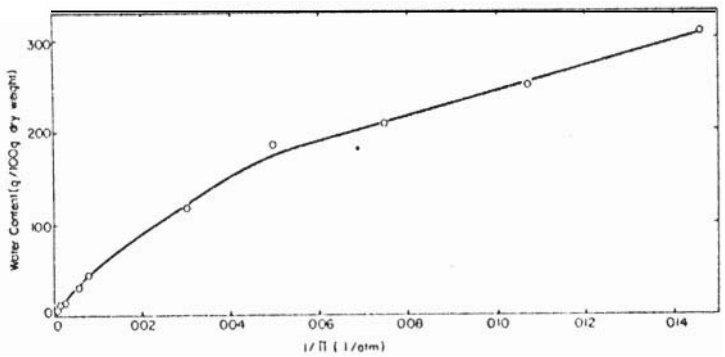

Figure 6. Equilibrium watcr content of frog sartorius muscles as a function of $1/\pi$. π is calculated from Equations 4 and 5 and is the osmotic pressure of the H_2SO_4 solutions the vapor of which the muscles are in equilibrium with. Data are the same as in Table 3. At $1/\pi = 0.0495$, $p/p^\circ = 0.985$, indicating the pronounced condensation of a wide range of water activities between 0 and 0.05 on the $1/\pi$ axis.

conventional method (p/p° above 0.985; $1/\pi$ above 0.0495).

In a higher osmotic pressure range the data no longer fall on the straight line that fits the data at lower osmotic pressure, but bend sharply downward toward the origin of the graph.

The slope of the straight line is such that by extrapolation, at $1/\pi = 0$. the water content is not zero as demanded by Equation 3. This recalls similar plots of $1/\pi$ against total cell volume. In the latter case tlic extrapolation departure from non-zero watcr content at infinite osmotic pressure was usually ascribed to osmotically inactive materials in the cells (e.g., oil droplets, proteins, and tlic so-called osmotically inactive watcr). In the present case, the total watcr content rather than total cell volume is plotted, one is left only with tlic explanation of osmotically inactive water amounting to $\frac{120}{400}$ = 30% of the total cell water.

What is osmotically inactive water? It would seem, by definition, it must be incapable of exerting any vapor pressure. Yet even ice, at 0°C, exerts considerable vapor pressure (i.e., 4.58 mm Hg, see 12).

Experimental results depicted in Figure 6, shows that no part of the water content has this property of osmotic inactivity. When the vapor pressure is very low, all water is lost from the tissue.

In conclusion, we must state that the data cannot be readily explained by the membrane theory.

The Model According to the Association-Induction Hypothesis

According to the association-induction hypothesis, the bulk of intracellular water exists in the form of polarized multilayers.[7,37] In a number of non-living protein-aqueous fixed charge systems (collagen, sheep's wool), it has already been shown that water uptake follows Bradley's adsorption isotherm:[7]

$$\log \frac{p^\circ}{p} = K_1^a \cdot K_3 + K_4 \qquad (6)$$

where a is the amount of water adsorbed in grams H_2O per 100 grams of dry weight, K_1, K_3, and K_4 are constant under specified conditions,[14] (see also 15).

Cope has shown on a theoretical basis that this model of cell also predicts the hyperbolic relation between external solute concentration and cell volume discussed above. This is valid, however, only in the range of external solute concentration low enough that Raoult's law is obeyed.[8] The present investigation deals with a much wider range of vapor pressure extending into areas where the hyperbolic relation is no longer followed.

Figure 7 shows the bulk of adsorbed water can be fitted with a Bradley adsorption isotherm but points at lower vapor pressures do not.

However, the full data can be much better described by the supposition that the water of frog muscle tissue consists of two fractions: the large fraction (a_1) constituting about 95% of the total water content of a resting muscle, follows the Bradley multilayer adsorption isotherm (see Fig. 8):

$$a_1 = 1.55 \times 10^2 \left\{ \log_{10} \left[(\log_{10} \frac{p^\circ}{p}) - 0.0014 \right] \right\} - 1.88 \times 10^2 \qquad (7)$$

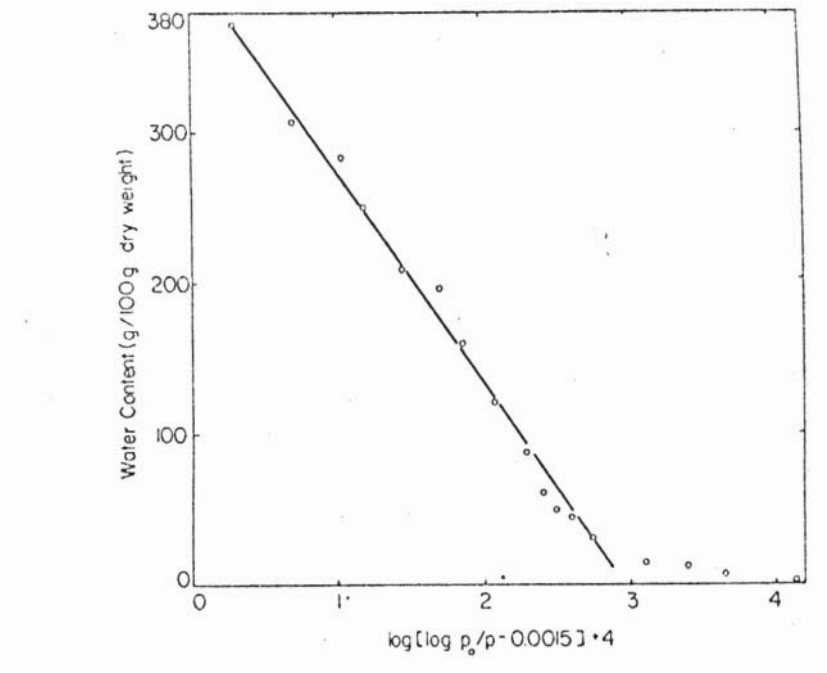

Figure 7. Equilibrium water content of frog sartorius muscles fitted to a single **Bradley adsorption isotherm. Data of Figure 4; plotted according to Equation 14.**

The smaller fraction (a_{II}), which is adsorbed more tightly, constitutes about 5% of the muscle water content. It is described by a Langmuir unimolecular adsorption isotherm:

$$a_{II} = \frac{[f]\,\tilde{K}\,(p/p^\circ)}{1 + \tilde{K}\,(p/p^\circ)}, \tag{8}$$

where [f], the total number of binding sites for this fraction, is that accommodating 15.6 grams or about 0.87 moles of water molecules per 100 g of dry cell matter. $\tilde{K}$, the adsorption constant is equal to 337.

The entire water content (a) is then the sum of a_I and :

$$a_I + a_{II} = 1.55 \times 10^2 \left\{ \log_{10} \left[(\log_{10} \frac{p^\circ}{p}) - 0.0014 \right] \right\} + \frac{5.29 \times 10^3\,(p/p^\circ)}{1 + 3.37 \times 10^2\,(p/p^\circ)} - 1.88 \times 10^2 \tag{9}$$

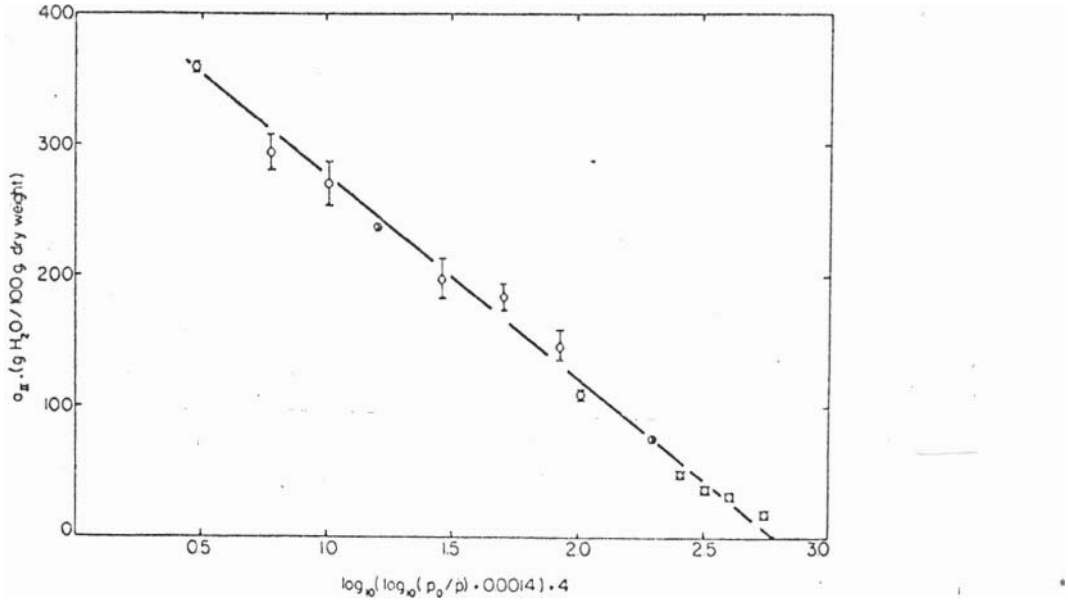

Figure 8. Multilayer adsorption plot of entire set of data after subtracting a small monomolecular adsorption.
The four lowest points which do not fit into the isotherm of Figure 7 can be fitted with a Langmuir adsorption isotherm (Equation 8). Subtracting from all points the theoretical values of watcr belonging to this Langmuir fraction (II), one obtains data shown above which fit equation 7.

In Figure 9, the equilibrium watcr content of sartorius muscle strips given in Table 3 is displayed. The line is calculated according to Equation 9.

The existence of one small, more tightly bound fraction dcscribcd by Equation 8 and a large, more loosely adsorbed fraction of intracellular water described by Equation 7, is in accord with conclusions from three other independent types of investigation:

(1) Studies of the diffusion of labeled water showed that two fractions of watcr exist in frog ovarian egg.[38] Neither one exhibits the propertics of normal liquid watcr. Again, the larger fraction exchanges its water more rapidly than the smaller fraction, which averages 10% of the total water content of the cells.

(2) Cope[3] concludcd from nuclear magnetic resonance (NMR) studies of D_2O in rat voluntary muscle and brain that the watcr in these cells exists in two fractions. In boih fractions the watcr is in a physical state different from that in normal dilute aqueous solution. The more tightly adsorbed fraction amounts to 27% of muscle water and 3% of brain water.

(3) Hazlewood, Nichols and Chamberlain[4] concluded from steady-state NMR studics that the watcr in rat muscles exists in two fractions. Both fractions have more structure than liquid water and the smaller, more tightly adsorbed fraction amounts to 8%.

The unanimity of the conclusions drawn from the four sets of experimental studies adds strong support to the concept that the physical state of water in living cells differs from that of water in dilute aqueous solution and that the bulk of intracellular water exists as polarized multilayers.[7,37]

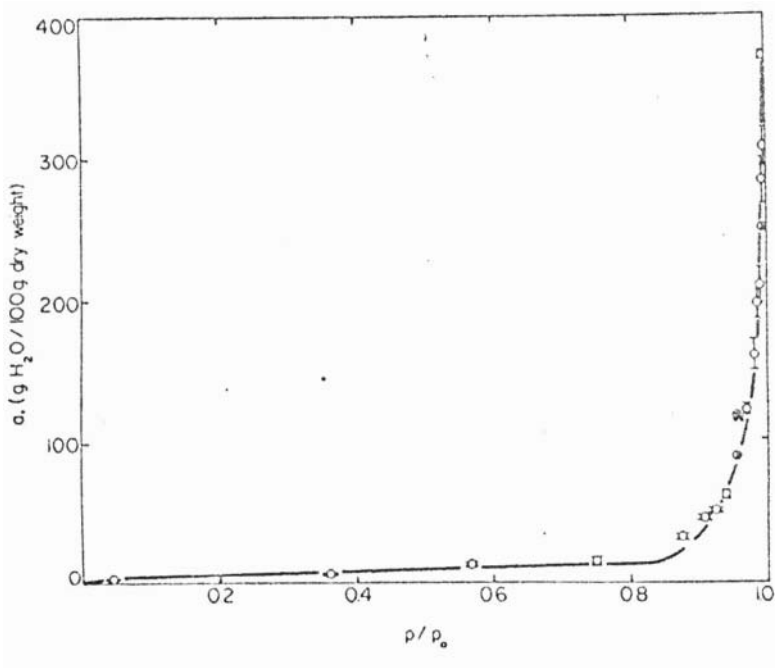

Figure 9. Uptake of water by frog muscle at various vapor pressures.
Points are experimental as given in Table 3. Solid line is theoretical, calculated according to Equation 9.

Cooperativity in Water Adsorption and Non-Swelling of Muscles in the Vapor Pressure of Hypotonic Solution

The concept of polarized multilayers of water embodies the concepts of indirect near-neighbor interaction and of (auto-) cooperative adsorption.[5,37] That is to say, adsorption and orientation of individual water molecules by cell proteins is not an independent process; rather, the orientation of one molecule influences the orientation of its neighbors by an inductive mechanism.[37,39] Auto-cooperative adsorption means that if one site sorbs water, its near-neighbor sites will tend to adsorb water more readily. At isotonicity, the system appears stabilized in a cooperative state and will refuse uptake of additional water until a much higher level of water activity is reached. In a normal swelling experiment this is produced by directly immersing the tissue in a medium of high water activity

(i.e., low salt concentration). In the vapor phase, on the other hand, the transport of water molecules onto the cell surface is such that the chance of the simultaneous arrival of a large number of water molecules at the same locus to effect a cooperative transition is small. The result is as we have observed: normal muscles either do not swell or swell to a smaller degree in a "hypotonic" vapor pressure, but when subsequently immersed in a hypotonic solution the same muscles took up additional amounts of water approaching those taken up by freshly isolated muscles.

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