Relative Humidity and the Killing of Bacteria: the Variation of Cellular Water Content with External Relative Humidity or Osmolality

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(Received 9 October 1961)

SUMMARY

The paper deals with the relationship between the water content of washed *Serratia marcescens* and the thermodynamic activity of water, a_w , over the range $0 < a_w < 1.0$. Two overlapping types of measurement are used: osmotic water exchange and water-vapour sorption. The data are given in terms of the equation $w = Bm^{-r}$ where w is water content, m is external osmolality (actual or effective, depending upon the technique used), B is a constant and r has values which can be interpreted in terms of the type of process involved: e.g. in the initial stages of osmotic dehydration, r of unity would indicate ideal solution behaviour; in the final stages of drying, any constant value of r would conform to an adsorption function like that of Halsey (1948). The data obtained by the two techniques used fall on a single curve with several zones including (a) non-ideal solution behaviour at high a_w , (b) two intersecting 'co-operative adsorption' zones at very low a_w , and (c) a region of variable r where processes such as dissolution of small molecules and 'saturated' swelling of interlinked polymeric structures are presumed to occur. It is thought that detailed interpretation of the unified water uptake curves would be valuable in understanding mechanisms of humidity-dependent loss of viability, in particular the zones of exceptionally rapid attenuation.

INTRODUCTION

This paper presents information on the water content of bacteria at external relative humidities (RH) from 100% to 'zero' as part of a study of humidity-dependent losses of viability. It was done partly to fill a need for a means of converting measured 'equilibrium' moisture content values for washed cells to the corresponding RH . Further, water transfer curves provide an overall picture which is useful when considering factors of possible significance in cell survival studies.

METHODS

Biological methods

Bacterial cell suspensions used in sorption measurements: five ml. portions of a 24 hr. broth culture of *Serratia marcescens* strain 8 UK were used to inoculate tryptose agar in Roux bottles. The cells were harvested in distilled water after

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24 hr. growth at 31°, centrifuged and resuspended in distilled water to give a suspension having about 10^{11} viable cells and 67 mg. dry residue/ml. Suspensions used for osmotic volume determinations: these were prepared from frozen cells stored at -20°, which had been grown in tryptose broth at 30° and harvested after 28 hr. by continuous process centrifuging. They were resuspended in an equal weight of growth medium and frozen in pellets. The pellets, when thawed, had a viable count of about 7×10^{11} /g.

The *Escherichia coli* B on which osmotic measurements were made was prepared from stock stored at -20°. The cells had been grown in nutrient broth at pH 8.1 and harvested at 18 hr. by continuous process centrifuging. After one washing with distilled water they were sealed into small tins and frozen. Before freezing, the total count was 5×10^{12} , the viable count about 3×10^{12} cells/g. dry solids. A single freezing and thawing cycle had negligible effect on viability.

Equilibration of bacterial cells with water vapour

The experiments were done with the silica spring sorption balance (McBain & Bakr, 1926) shown in Fig. 1. Freeze-drying and subsequent equilibration with water vapour took place within the same sealed chamber under precisely controlled conditions.

Procedure: (a) Freeze-drying of cells: 1.5 ml. of stock suspension was placed in an aluminium foil bucket and frozen in a closed vessel immersed in solid CO₂ and alcohol for 30 min. The cold trap in the sorption apparatus, containing water, was immersed in solid CO₂ and alcohol. The spring suspension frame, spring, and frozen sample were placed in position (Fig. 1) and the apparatus evacuated. Drying was continued to constant weight, 16 hr. being sufficient as a rule. (b) Sorption: the vacuum line was closed and the water reservoir warmed in stages, sufficient time being allowed at each reservoir temperature for constant weight to be reached. At the lower water-vapour pressures this took about 1½ hr., increasing at higher pressures to about 6 hr. because of 'collapse' of the fluffy freeze-dried material and also because of the larger absolute amounts of water to be transferred. Constancy of weight, once reached, was maintained for at least 40 hr. (c) Reversibility: loss of water by the rehydrated freeze-dried samples was followed by reversing the sequence of changes of reservoir temperature. (d) Test of performance: the water sorption curve of a specimen of solid egg albumin (not dissolved and freeze-dried) followed very closely the course described by Bull (1944).

Measurement of water inaccessible to solutes

Total cell volume V (Fig. 2) was determined by measuring the dilution of a solution of dextran by the intercellular water. Dextran concentrations and leakage from the cells were determined refractometrically (Brice & Halwer, 1951).

Osmotic water transfer was determined by measuring that portion of a cell pad which was inaccessible to sucrose or phosphate added in sufficient quantity to bring about the desired water movement. In two experiments sucrose and phosphate were added simultaneously, and concordant values for the inaccessible cell volume were obtained from independent analyses for the two substances. The experimental procedure was as follows: cell pads of volume V_c containing g gram of dry cells were prepared by centrifuging washed cells (10 ml. distilled water to 1 g. wet cells)

at 10,000 g. Each pad was resuspended in volume V_a of a solution of concentration c_a of one of the above solutes. The bacteria were removed by centrifuging and the supernatant of concentration c_f , containing the original added solution diluted by water in the cell pad, was decanted. In absence of leakage of intracellular solutes, the ratio c_a/c_f would be simply related to the amount of water in the pellet available to dilute the added solution. There was always a little leakage, and control runs were done by adding V_a ml. of water to another cell pad. In separate experiments

Fig. 1. Silica-spring sorption balance used to measure equilibration of bacterial cells with water vapour. Bacterial sample is in aluminium foil bucket. Changes in weight of the sample are measured by observing movement of the bucket with respect to a thin reference wire on the spring suspension frame, using a travelling microscope independently mounted. Changes of 0.03 mg could be detected. Absolute vapour pressure of water p above sample is determined by temperature of water or ice in cold trap at bottom of apparatus, while relative vapour pressure or water activity is determined by the ratio of p to the saturation vapour pressure of water p_0 at the temperature of the jacket surrounding the sample chamber. Direct thermal exchange between sample and cold trap is minimized by having constant temperature water flowing *downward* through jacket, and by a reflecting disk below sample.

it was found that the leakage of substances containing phosphate from the bacteria was a function of the ionic strength of the environment. Thus when phosphate was being used to measure inaccessible volume, the control run was done with a KCl solution of initial concentration c_a instead of pure water. If the concentration c_0 of the supernatant from the sample represents the increment in c_f caused by leakage, the true dilution factor is $c_a/(c_f - c_0)$ and the volume inaccessible to the solute in question, V' ml./g. dry cells, is given by

$$gV' = V_c + V_a(c_f - c_0 - c_a)/(c_f - c_0). \quad (1)$$

For dextran, c_0 varied from 0.08 to 0.5 c_f as the salt concentration was increased; for sucrose, c_0 varied from 0.06 to 0.08 c_f as the sucrose concentration was increased; for phosphate, c_0 was 0.01 to 0.06 c_f .

Sucrose analyses were made in the differential refractometer at 5461 Å. Phosphate was determined by the Fiske-SubbaRow method as modified by Boltz & Mellon (1947). In converting the analytical data for the equilibrium continuous phase to external osmolalities, use was made of the osmotic coefficient values of Stokes (1945) for phosphate and Scatchard, Hamer & Wood (1938) for sucrose.

THEORY

The cell model

Representing the bacterial cell by an osmotically sensitive protoplast and cell wall, the volume subdivisions are portrayed in Fig. 2.

Fig. 2. Volume subdivisions of bacterial cell (not to scale). Upper left, fully hydrated cell immersed in solution of osmotic pressure Π_e . Upper right, cell partly dehydrated osmotically. Lower left, fully hydrated cell free of extracellular liquid equilibrated with water vapour at partial pressure p_1 close to saturation. Lower right, cell partially dehydrated by equilibration with water vapour at partial pressure p equivalent osmotically to the external solution used to produce osmotic dehydration in upper right. In all diagrams horizontal dots represent cytoplasmic membrane and black areas are cell solids of total weight 1 g. Solids above membrane belong to cell wall, those below membrane to cell interior. The corresponding volumes of solids and aqueous subdivisions are shown on the left and are self-explanatory. In upper left diagram, the wide area above the 'organism' represents a suspending medium containing dextran, D , sucrose, S , and water, W . The 'gateway' to the cell wall is large enough to admit molecules of S but D is excluded. The gateway to the osmotically sensitive part of the organisms, the channel to the right of the cell solids, will not admit S or D but is freely permeable to W . The symbols in the middle, $\Delta(V-v_i)$ and $\Delta(V-v)$, represent the volume changes actually measured by osmotic water distribution measurement and by water sorption measurements, respectively.

Notation

The following symbols will be used in addition to those in Fig. 2.

a , constants in the BET, Halsey, and logarithmic isotherms; a_w , water activity or relative vapour pressure p/p_0 ; B , constant in equation (18); c , constants in the

BET and logarithmic isotherms; d , density of water; f , a factor used in comparing water sorption data with osmotic volume changes, equation (16); m , molality, moles/1000 g. water. Subscript e refers to external medium, i to phase within cell osmometer; m' , osmolality, $\nu\phi m$ for electrolytes, ϕm for simple non-electrolytes; M_w , molecular weight of water; n_i , moles of osmotically active solute in osmotically sensitive volume V_i (Fig. 2); p , p_0 , vapour pressures of water over an aqueous solution and over pure water, respectively; r , mole ratio; moles of a solute per mole of water (equation 6), r , a constant in Halsey's isotherm and in equation (18); R , gas constant, 8.3144 joule deg.⁻¹ mole⁻¹; RH , relative humidity, 100 a_w ; T , absolute temperature; w , water content of washed bacteria equilibrated with a vapour phase, g./g. dry cells; θ , fraction of water adsorption sites occupied by water; Π , osmotic pressure; ρ , density of dry bacterial cell solids; ϕ , molal osmotic coefficient of solvent, equations (2), (7); ν , number of ions into which a strong electrolyte dissociates.

Osmotic water transfer

The osmotic pressure equations (see Dick, 1959) for the osmotically responsive portion of the cell and for the ambient solution of reference substance, respectively, are

$$\Pi_i(V_i - v_i) = n_i \phi_i RT, \quad (2)$$

$$\Pi_e = m_e \phi_e dRT/1000 = m'_e RT/1000. \quad (3)$$

Assuming osmotic equilibrium between the interior and exterior of the osmometer and the absence of any hydrostatic pressure difference,

$$m'_e = m'_i, \quad (4)$$

$$\log(V_i - v_i) = \log \phi_i n_i - \log m'_e + 3. \quad (5)$$

Ideal behaviour would be represented by a straight line of unit slope, while lines of any other slope or of varying slope would indicate variation of $\phi_i n_i$ with external osmolality, failure to achieve osmotic equilibrium, or intrusion of a hydrostatic pressure term.

A sorption isotherm equivalent to equation (5)

A sorption isotherm is usually a plot of water content w against water activity a_w . In order to describe the whole course of cell water exchange a convenient parameter is the osmolality m'_e since this can be given a formal value for any water activity by way of Bjerrum's definition of the osmotic coefficient (Guggenheim, 1957)

$$\ln a_w = -\phi \Sigma r. \quad (6)$$

It follows that

$$\ln a_w = -10^{-3} \phi m M_w = -0.01806 m'_e \text{ and } m'_e = -127.83 \log a_w. \quad (7)$$

The description of sorption data by an equation equivalent to (5) would be the same as using an isotherm of the form

$$\log(V - v) = A - \log(-\log a_w), \quad (8)$$

$$A = \log \phi_i n_i + 0.8984. \quad (9)$$

A modified equation (5) for sorption data

The so-called logarithmic adsorption isotherm (De Boer, 1956)

$$\ln a_w = \theta/c'RT \quad (10)$$

has been widely used for chemisorption, while for physical adsorption Halsey's (1948) equation can be written

$$\ln a_w = -a/RT\theta^{1/r}. \quad (11)$$

Using the subscript 1 to mean completion of a monolayer of water ($\theta = 1$), we can put

$$-a/RT = \ln(a_w)_1 \quad \text{and} \quad \theta = (V-v)/(V-v)_1 \quad (12)$$

giving

$$\ln a_w = \theta^{-1/r} \ln(a_w)_1 \quad (13)$$

or, from (7),

$$m'_e = \theta^{-1/r} (m_e)_1 \quad (14)$$

and

$$\log(V-v) = -r \log m'_e + r \log (m'_e)_1 + \log(V-v)_1. \quad (15)$$

The corresponding equation for osmotic volume change is (5), which can be applied to the water volume of the whole cell by assuming

$$V-v = f(V_i-v_i) \quad (16)$$

so that

$$\log(V-v) = -\log m'_e + \log \phi_i n_i + \log 1000 f, \quad (17)$$

where ϕ_i is a function of m'_e . Comparing (15) and (17),

$$\log(V-v) = -r \log m'_e + \log B, \quad (18)$$

$$B = (m'_e)_1^r (V-v)_1, \quad (19)$$

$$\phi_i n_i = B m_e'^{a-r} / 1000 f. \quad (20)$$

Equation (18) is applicable wherever a straight line is obtained by plotting $\log(V-v)$ against $\log(m'_e)$. With unit slope ($r = 1$), ideal solution behaviour is indicated; otherwise, $\phi_i n_i$ varies according to equation (20). In the 'osmotic' region this can be attributed to increase of osmotic coefficient with increasing m'_e if $r < 1$, rather than to a change of n_i . If the line is curved, equation (18) does not hold, because (a) the functional dependence of ϕ_i upon m'_e does not follow equation (20), or (b) n_i varies with m'_e because of leakage, precipitation, or dissolution, or (c) a hydrostatic term is omitted. The behaviour of r in the sorption region is discussed later.

Application of equation (18) to inaccessible volume data

The 'osmotic' cell volume V' is measured by a dilution technique. It comprises water within the osmometer and the entire dry volume of the cell, and reference to Fig. 2 will show that (V_i-v_i) is not directly comparable to $(V-v)$. To reconcile osmotic and sorption data, the assumption is made (equation 16) that the water fraction and osmotic coefficient are the same for the osmotically responsive part of the cell as for the whole cell. The factor f can be obtained from dextran and sucrose impermeable volumes for standard conditions (subscript s); by inspection of Fig. 2,

$$f = (V_s \rho - 1) / (V'_s \rho - 1). \quad (21)$$

RESULTS

Sorption experiments

A sorption cycle taken at 20° is illustrated in Fig. 3; the characteristic hysteresis will be noted. Any progressive change in the specimen during the lengthy experiment was ruled out by checking a few individual points with fresh cell samples. The points at the highest *RH* values (p/p_0 around 0.99) are of course of little significance, for maintenance of an *RH* of 99% at 20° in these experiments depended upon maintaining a steady temperature difference between the main chamber and reservoir of only 0.15°. The results of experiments at 36° resemble those at 20° in accuracy and general appearance. The data refer to essentially non-viable cells; the fractional viable recovery after freeze-drying was about 10^{-1} , that at the end of a 14-day experiment about 10^{-10} .

Fig. 3. Water sorption and desorption curves of *Serratia marcescens* at 20°. Abscissa, relative aqueous vapour pressure or water activity. Ordinate, grams sorbed water per gram dry weight of organisms. Open circles, water uptake; small heavy circles, water loss.

The data conform over the usual range (*RH* 10 to 45%) to the BET equation (Brunauer, Emmett & Teller, 1938)

$$a_w/w(1-a_w) = (1+ca_w-a_w)/ac. \quad (22)$$

Values of the constants a and c are given in Table 1.

Osmotic shrinkage of cells in sucrose and phosphate solutions

The dilution measurements gave values of the cell volume inaccessible to the added solute, V' ml./g. dry solids. The cell water of *Serratia marcescens* and *Escherichia coli* B inaccessible to the solute was taken to be $V' - 0.763$, assuming the density of the dry residue to be 1.31. This was converted by equation (16) into

Table 1. *BET constants* for bacteria and proteins*

See text equation (22). The constant a is usually interpreted as the weight of a complete monolayer of water in grams per gram dry material. The constant c is dimensionless and is related exponentially to the heat of adsorption of the water monolayer expressed in multiples of RT .

		D1		S1		D2	
		<i>a</i>	<i>c</i>	<i>a</i>	<i>c</i>	<i>a</i>	<i>c</i>
<i>Serratia marcescens</i>	20°	—	—	0.069	10.5	0.069	52.4
<i>Pseudomonas aeruginosa</i> †	30°	0.064	35.5	—	—	—	—
<i>Staphylococcus aureus</i> †	30°	0.054	48.5	—	—	—	—
		<div> <i>a</i> <i>c</i> </div>					
Egg albumin‡		0.0615 11.60					
Stretched nylon‡		0.0176 5.97					
Collagen‡		0.0952 17.80					

* Brunauer, Emmett & Teller (1938).

† Imelik (1951), desorption of wet cells.

‡ Bull (1944), average of adsorption and desorption at 25 and 40°.

D1 indicates first desorption of wet cells. S1 is the first sorption of water by freeze-dried cells and D2 is the succeeding desorption.

Fig. 4

Fig. 5

Fig. 4. Water volume of one gram dry *Serratia marcescens* as a function of environmental osmolality. Lower scale of abscissae: actual or effective external osmolality. Upper scale, water activity or relative humidity $\times 1/100$. In osmotic shrinkage experiments the actual external osmolality is determined by the nature and concentration of added solutes, and the recorded water volumes are corrected for the presence of the cell wall, which does not participate in the osmotic shrinkage (see text). In water sorption experiments the cell water is measured directly as a function of water activity in the vapour phase, and the corresponding effective osmolalities are calculated by text equation (7). Open circles: data obtained with added sucrose. Half-closed circles: with added sodium dihydrogen phosphate. Closed circles: sorbed water.

Fig. 5. Water volume of 1 g. dry *Escherichia coli* as a function of environmental osmolality. Open circles: data using added sucrose; closed circles: added sodium dihydrogen phosphate. See also legend to Fig. 4.

total cell water, $(V-v)$, per g. dry residue, using for V , the volume inaccessible to dextran (for *S. marcescens*, 3.38 and for *E. coli* 2.65 ml./g.) and for V' , the volume inaccessible to sucrose (for *S. marcescens*, 2.49, for *E. coli* 2.00 ml./g.). Application of equation (21), with ρ equal to 1.31, gives $f = 1.52$ for both cell types.

Combined osmotic and sorption data

The data for *Serratia marcescens* are given in Fig. 4. There appear to be three distinct regions of approximate conformity to equation (18), for which the appropriate values of the 'constants' r and B are assembled in Table 2. The osmotic shrinkage data for *Escherichia coli* are similarly plotted in Fig. 5 while the corresponding numerical data are included in Table 2.

Table 2. 'Constants' and limits of linear water exchange curves of bacteria

See text equations (18) and (20) and Fig. 4. Column (1) identifies the linear region in Fig. 4, corresponding to slopes given in column (4). These zones extend over the range of external osmolalities in column (3) and the corresponding ranges of water volume are shown in column (2). Column (5) gives the values of the 'constant' B used in calculating the osmotic coefficients ϕ_i of column (7) and the apparent number of moles of osmotically active intracellular solutes per gram.

(1) Zone	(2) $(V-v)$ ml./g. dry cells	(3) m'_o	(4) r	(5) B	(6) $10^3 n_i$	(7) ϕ_i
<i>Serratia marcescens</i>						
1, monolayer sorption	0.02-0.05	280-100	1.20	16	—	—
3, multilayer sorption	0.1-0.5	53-4	0.60	1.07	—	—
5, osmotic shrinkage	1.0-2.2	0.46-0.1	0.82	0.44	—	—
	0.8	0.46	0.82	0.44	0.207	1.23
	2.2	0.145	0.82	0.44	0.207	(1.00)
<i>Escherichia coli</i>						
5, osmotic shrinkage	0.8-1.5	3.0-0.18	0.55	0.57	—	—
	0.3	3.0	0.55	0.57	0.176	3.54
	1.5	0.18	0.55	0.57	0.176	(1.00)
<i>E. coli</i> *						
5, osmotic shrinkage	1.2-3.55	3.0-0.3	0.50	2.2	—	—
	1.2	3.0	0.50	2.2	0.80	3.16
	3.55	0.3	0.50	2.2	0.80	(1.00)
<i>Staphylococcus aureus</i> *						
5, osmotic shrinkage	0.76-2.24	3.0-1.0	1.00	2.24	1.49	1.00

* Calculated from data of Mitchell & Moyle (1956). See text.

DISCUSSION

Continuity of osmotic and sorption data. Figure 4 represents both osmotic shrinkage and water sorption of washed *Serratia marcescens*. Since there is no discontinuity, our assumption that the water relationships of the osmotically responsive portion are not greatly different from those of the organism as a whole is probably a reasonable one. The compatibility of the two sets of data also suggests that at the highest humidities equilibrium was attained, despite the difficulty of maintaining nearly saturated atmospheres (Ashpole, 1952).

Zones of water transfer. The following zones can be recognized in Fig. 4: (1) water monolayer formation; (2) transition to multilayer adsorption; (3) multilayer water uptake; (4) transition to osmotic swelling; (5) osmotic swelling; (6) transition to constant cell volume; (7) limiting cell volume.

Zone (1). Formation of a monolayer. This is a steep linear region terminating when about 0.063 g. water has been taken up per g. dry cells. Similar initial phases have been noted in other processes, for example, in the adsorption of water on anatase (Halsey, 1948). The inflexion presumably signals completion of a monolayer of water, since the value 0.063 agrees quite well with the BET constant a (Table 1). Similar values of a have been obtained by Imelik (1951) for other micro-organisms (Table 1). They are fairly typical of an average protein; indeed our *Serratia marcescens* curves are scarcely distinguishable from that of egg albumin. It is therefore likely that all the cell solids are accessible to water vapour and that the sorption curves at low RH values are essentially those of the bacterial protein and nucleic acid which together comprise perhaps 90 % of the dry cell mass. If there are significant contributions from proteins of more extreme types, such as collagen and silk, these must average out. The sorbed monolayer is not readily removed (Fig. 3) as seen in the different BET constants c for sorption and desorption (Table 1), the latter having values comparable to those of Imelik (1951) for the initial drying of wet cells.

Zones (2) and (3). Multilayer adsorption. After a short curved transition, there is a straight line of reduced slope ($r \sim 0.6$) which extends to a_w about 0.92. This covers the range of the cases illustrated by Halsey (1948) and interpreted by him in terms of co-operative (multimolecular) adsorption upon a non-uniform surface.

Zone (4). Transition from co-operative adsorption to osmotic swelling. With continued application of equation (18) θ would approach ∞ , with gross condensation of water. Instead, the slope r decreases, perhaps because of an intermolecular structure which limits the expansion of the cell solids. This would be equivalent to a decrease in the effective area of adsorbent.

This region of the sorption curve overlaps the curve of osmotic dehydration. Evidently, at RH values high enough to distend the hydrophilic cell structures to their maximum extent, some or all of the small molecular solutes have dissolved, and the further course of events with increasing RH is best considered in terms of the osmotic coefficient ϕ_i . As the RH increases from 80 to 99, the slope continues to decrease somewhat, with an average r of about 0.21. Thus the variation of $n_i\phi_i$ with external osmolality follows equation (20) roughly, increasing about 12-fold as m'_e increases from 0.5 to 12.5. Since some solutes may be precipitated at the lowest RH values within this region, the osmotic coefficient must increase by a larger factor than 12 and the increase must be due mainly to interactions involving the polymeric components and the non-solvent water (hydration).

Zones (5) to (7). Limited osmotic swelling leading to constant cell volume. When $m'_e \sim 0.6$ there is a fairly abrupt transition to a third linear region with $r \sim 0.82$. This is the region of osmotic swelling. The behaviour deviates appreciably from the Boyle-Van't Hoff equation (Lucké & McCutcheon, 1932), with r differing from unity. If the osmotic coefficient is unity at the 'dilute' end of this linear zone, then it rises to 1.23 when the external osmolality is increased to 0.46 (Table 2).

If equation (18) were followed at external osmolalities below 0.1, the line in Fig. 4 would be extended until lysis occurred, perhaps with a shift in slope towards ideal behaviour ($\phi_i = 1$). *Serratia marcescens* and many other bacteria, however, are not lysed even by distilled water. Fig. 4 shows that there is a rather sharp

transition from osmotic swelling to the maintenance of large internal hydrostatic pressures at constant volume.

The existence of the zone of constant cell volume was deduced by Mitchell & Moyle (1956) from values of the volume of *Escherichia coli* inaccessible to inorganic phosphate in presence of different concentrations of NaCl. The transition appeared to commence when m'_e was about 0.4, but this conclusion rested upon a single experimental point; a recalculation shows that equation (18) is followed throughout, with $r \sim 0.5$ (Fig. 6). Our own measurements on *E. coli* (Fig. 5), agree with the recalculated Mitchell & Moyle results and show also that the external osmolality must be reduced to about 0.18 before the slope of the line begins to fall off. The cell volume is constant when m'_e is less than 0.1.

Fig. 6

Fig. 7

Fig. 6. Water volume of one gram dry *Escherichia coli* as a function of environmental osmolality, from volume measurements in NaCl solutions using inorganic phosphate as tracer. Values of phosphate impermeable volume estimated from Mitchell & Moyle's (1956) graph, their Fig. 4, and corrected for the fact that the cell wall contributes to the dry weight but does not participate in the osmotic movement of water. The correction factor f (equation 16) was taken to be 1.52, determined as described in this paper.

Fig. 7. Water volume of one gram dry *Staphylococcus aureus* at different relative humidities. Data of Mitchell & Moyle (1956), given in their Fig. 1 as a plot of weight of cell sample against reciprocal of the molality of the sucrose solution used to control the RH . Values read from this curve were corrected for dry weight of cell sample, taken as 47 mg, and for osmotic coefficient of water in sucrose solutions (Scatchard *et al.* 1938).

Limiting cell volume in vapour phase equilibration. In vapour phase equilibration of osmotically resistant cells, the increase of internal hydrostatic pressure must always be such as to bring the vapour pressure of the cell into equilibrium with the atmosphere; the water content must remain nearly constant until the atmosphere becomes fully saturated. At this point the cell is in equilibrium with both liquid water and water vapour and the equilibrium water content of the system is indeterminate. In the presence of a trace of external solute, however, it is infinite, and uptake of water in a saturated or slightly undersaturated atmosphere will proceed indefinitely. The predicted levelling-off of water content in vapour phase equilibration has been found for *Staphylococcus aureus* by Mitchell & Moyle (1956), starting when m'_e is about 0.6 (Fig. 7). At lower RH values there is, as with *Serratia marcescens*, an ideal region succeeded by one of increasing osmotic coefficient.

Solute content of bacteria. The values of n_i (Table 2) indicate the amounts of

osmotically active solutes per g. dry cells, while the values of m'_e at the limiting (maximum) cell volume give the internal osmolality. It is of interest that Mitchell & Moyle's *Escherichia coli*, grown in a salt-glucose medium, contained four times as much dissolved substance as ours, grown in nutrient broth of low salt content. *Staphylococcus aureus* contains much more dissolved material than *E. coli* or *Serratia marcescens*—1.49 m-mole/g., and a limiting internal osmolality of 1.0. It is surprising that the apparent osmotic coefficient remains constant over a three-fold range of m'_e (1.0 to 3.0).

Relative humidity and the stability of air-borne bacteria. At several points the interpretation of the water uptake curve of *Serratia marcescens* is speculative and at others incomplete. Nevertheless, there is relevance to the problem of stability of air-borne bacteria: (1) The existence of inflexions in the curve invites more detailed interpretation and comparison with stability data (Monk, McCaffrey & Davis, 1957). (2) The hysteresis-like retention of water during desorption suggests that bacterial stability may depend upon the history of the particle, especially upon the direction of water transfer. A more detailed study might reveal slow irreversible changes in the dehydrated organisms or the existence of potential energy barriers, e.g. supersaturation, failure to dissolve, formation of cross-linked structures which do not expand reversibly on wetting, capillary effects of the type discussed by Hill (1952). R. M. Izatt & T. Hatch (to be published) have shown clearly the effects of relatively gross amounts of hygroscopic additives in modifying the water uptake curves. (3) Clearly the curve of Fig. 4 does not represent a series of true equilibria, for metabolic processes continue (Monk *et al.* 1957) and viability is gradually lost. Despite the enormous retardation of these processes, however, there must be zones of water content within which the diffusion rates of intracellular molecules or polymeric submolecules become sharply dependent upon *RH*; one such region presumably would be within the overlapping zones of non-ideal solution and of failing co-operative multilayer adsorption. Here one might well seek special biological effects, arising perhaps from random translocations of specific molecules present in statistically small numbers. (4) Comprehensive data such as those in Fig. 4 are required in any theoretical study of air-borne organisms since rates of 'equilibration' with an atmosphere will depend partly upon the amounts of water to be transferred for a given change of *RH*. The differential amounts, dV/da_w , vary by more than 2 orders of magnitude between *RH* 0 and 100 %.

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