On the Mechanism of Fluid Exchange of Tissues *in vitro*

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There have been many studies in which hydration of mammalian tissues has been determined after incubation in saline media under varying conditions of metabolism (reviewed by Robinson, 1953). The results indicate that when metabolism is inhibited, various tissues swell in saline media unless the osmotic concentration is greater than about twice that of extracellular fluid (Opie, 1949; Robinson, 1950; Aebi, 1953). When tissue metabolism is active the changes in hydration are much less (Stern, Eggleston, Hems & Krebs, 1949; Robinson, 1950; Aebi, 1952*b*). These findings have been interpreted as evidence that the intracellular fluids are normally hypertonic with respect to their extracellular fluid environment and that this hypertonicity is maintained by active metabolic processes (Robinson, 1950, 1953; Robinson & McCance, 1952; Bartley, Davies & Krebs, 1954).

The validity of such an interpretation is entirely dependent upon the assumption that net movements only of water occur across the cell membranes during such experiments *in vitro*. If net movements of osmotically active substances occur as well, observed changes in hydration cannot be used as evidence for the existence of differences between the total solute concentrations of intra- and extra-cellular fluids of metabolizing tissues. The present results demonstrate that large net shifts of ions do in fact occur across cell membranes when metabolism is inhibited. A different explanation of the changes in tissue hydration is presented which is based upon the known dependence on metabolism of ionic gradients in tissues.

EXPERIMENTAL

Studies were made on slices of guinea-pig kidney cortex, rat cerebral cortex and rat liver. The outermost slice of tissue was rejected. Slices approximately 0.3 mm. thick were spread on hardened filter paper (Whatman no. 54) to remove surface blood, urine and cellular debris. Kidney cortex slices were incubated in a bicarbonate-saline solution similar to medium III of Krebs (1950) but without the addition of the organic substrates. To each 2 ml. of medium gassed with $O_2 + CO_2$ (95:5) was added 0.1–0.2 g. of tissue.

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The flasks were kept at 0° to inhibit metabolism and were shaken in a Dubnoff Metabolic Shaking Incubator (Precision Scientific Co., Chicago, U.S.A.) for 30 min. The slices were reblotted and then weighed.

Slices of liver and cerebral cortex were similarly treated except that the medium used was a mixture of sodium and potassium chlorides containing 160 m-equiv. of Na and 5 m-equiv. of K/l. Because of their friability slices of cerebral cortex could not be blotted on filter paper. Excess of moisture was removed by repeatedly touching the slices against a clean glass plate. The control slices were similarly dried after momentary immersion in the incubating medium.

The water content of the slices was obtained as the difference between their weights before and after drying overnight at 105°. The tissue electrolyte determinations were made on an extract obtained by digesting the tissue specimen for at least 24 hr. at room temperature in 5.0 ml. of 0.1N-HNO₃. This method of digestion avoids loss of chloride by volatilization or by conversion into organic chloro compounds (Shenk, 1954). Chloride of tissues and media was analysed by the method of Sanderson (1952). Na and K concentrations of tissue and supernatant fluid were determined with a Beckman DU Spectrophotometer with flame-photometer attachment. Portions of the dilute acid extract or supernatant fluid were further diluted to give final concentrations of Na and K of approximately 0.15 m-equiv./l. Na and K were added to yield final concentrations of 5 m-equiv./l. in the samples analysed for potassium and sodium respectively.

RESULTS

Table 1 shows the effects on the water, sodium, chloride and potassium contents per kilogram of tissue solids of incubating fresh slices of guinea-pig kidney cortex at 0° for 30–50 min. The changes in water and electrolytes are all statistically significant. The tissue swelling and reciprocal changes in sodium and potassium content have been extensively discussed by previous workers, but of special interest is the large gain in tissue chloride that occurred. For the increase of 0.94 l. of water by the swollen tissue a concomitant increase of 145 m-equiv. of chloride took place. The chloride concentration in the increment of water gained may be said to equal 154 m-equiv./l. whereas the incubation medium had a concentration of chloride of from 130 to 140 m-equiv./l. It may be further calculated that some 145–170 m-equiv. of sodium entered the tissue in accompaniment with this

gain in tissue chloride, the amount depending upon the degree to which penetration of other anions beside chloride (phosphate and bicarbonate) of the medium took place. Of the 229 m-equiv. of sodium that entered the tissue there thus remains some 60–85 m-equiv. which entered the tissue in net exchange for potassium. Exact equality between this increment of sodium gained and the potassium lost by the tissue cannot be expected because of the likelihood of some loss of diffusible anions from the cells. It is apparent from these results that the increase of tissue sodium considerably exceeds the loss of tissue potassium, and that the swelling process may be considered as a gain by the tissue of approximately isotonic medium.

140 m-equiv./l. Thus the tissue swelling may be said to result from an entry of approximately isotonic medium into the tissue when metabolism is interfered with by any of the means tested.

Table 3 shows that the swelling of cerebral cortex and liver slices *in vitro* involves similar ion movements into these tissues. The calculated chloride concentration in the water gained was 225 and 231 m-equiv./l. for cerebral cortex and liver slices respectively. The concentration of chloride in the medium was 165 m-equiv./l. The discrepancy between these values is in the same direction but larger than that seen in results presented so far and will be discussed below. After subtracting the equivalent quantity of sodium which entered the

Table 1. *Changes in the sodium, potassium, chloride and water contents of guinea-pig kidney cortex slices on incubation at 0°*

Slices of tissue (0.1–0.2 g.) incubated 30–50 min. in 2.0 ml. of bicarbonate-saline, pH 7.4, gas phase O₂ + CO₂ (95:5). Mean values $\pm$ S.E.

	No. of samples	Dry wt. (%)	Water per kg. dry solids (kg.)	m-equiv. per kg. dry solids		
				Na	Cl	K
(a) Before incubation	14	23.7 $\pm$ 0.35	3.22 $\pm$ 0.04	350 $\pm$ 16	272 $\pm$ 7	365 $\pm$ 8
(b) After incubation	14	19.4 $\pm$ 0.29	4.16 $\pm$ 0.05	579 $\pm$ 18	417 $\pm$ 17	240 $\pm$ 7
<i>b - a</i>	—	—	+0.94 $\pm$ 0.06	+229 $\pm$ 24	+145 $\pm$ 18	-125 $\pm$ 11

Table 2. *Changes in the sodium, potassium, chloride and water contents of guinea-pig kidney cortex slices on incubation at 37°, at 0°, with 0.2 mM 2:4-dinitrophenol (DNP), and anaerobically, mean values $\pm$ S.E.*

Data recalculated from Whittam (1954).

	No. of samples	Temp. at incubation °	Aerobic or anaerobic	Dry wt. (%)	Water/kg. dry solids (kg.)	m-equiv. per kg. dry solids		
						Na	Cl	K
(a) Before incubation	16	—	—	22.3 $\pm$ 0.2	3.49 $\pm$ 0.02	284 $\pm$ 9	229 $\pm$ 36	342 $\pm$ 4
(b) After incubation	23	37	Aerobic	20.3 $\pm$ 0.3	3.93 $\pm$ 0.05	444 $\pm$ 9	277 $\pm$ 28	370 $\pm$ 8
(c) In cold	12	0	Aerobic	17.0 $\pm$ 0.5	4.89 $\pm$ 0.12	659 $\pm$ 17	460 $\pm$ 34	182 $\pm$ 4
(d) With DNP	—	37	Aerobic	17.0	4.89	730	454	204
(e) Anaerobic	18	37	Anaerobic	17.3 $\pm$ 0.3	4.78 $\pm$ 0.07	660 $\pm$ 13	455 $\pm$ 28	159 $\pm$ 6
<i>b - a*</i>	—	—	—	—	+0.44 $\pm$ 0.05	+160 $\pm$ 13	+48 $\pm$ 46	+28 $\pm$ 9
<i>c - a</i>	—	—	—	—	+1.40 $\pm$ 0.14	+375 $\pm$ 19	+231 $\pm$ 50	-160 $\pm$ 6
<i>d - a</i>	—	—	—	—	+1.40	+446	+225	-138
<i>e - a</i>	—	—	—	—	+1.29 $\pm$ 0.08	+376 $\pm$ 16	+226 $\pm$ 46	-183 $\pm$ 7

* The increase in sodium found on incubation at 37° with α -oxoglutarate as substrate was shown by Whittam & Davies (1953) to be correlated with an accumulation of substrate in the tissue. This accumulation of sodium oxoglutarate in the tissue may account for the small increase in tissue hydration without significant increase in tissue chloride.

Table 2 shows similar results which have been obtained by recalculation of data from Whittam (1954). The effects of oxygen lack and of 2:4-dinitrophenol, as well as of incubation at 0°, are shown. Significant increases in tissue water, sodium and chloride with loss of potassium are again noted (*c - a*, *d - a*, and *e - a*). The gains in tissue chloride were sufficient to yield calculated chloride concentrations in the increments of water of 165, 160 and 175 m-equiv./l. with chilling, 2:4-dinitrophenol, and anaerobiosis respectively. The chloride concentration of the medium was 130–

tissue with chloride, there remain 179 and 120 m-equiv. to exchange for the observed potassium loss of 257 and 178 m-equiv. from cerebral cortex and liver slices respectively. As chloride was the only anion in the medium, 78 (257 – 179) and 58 (178 – 120) m-equiv. are estimates of the loss of diffusible anions from the cells of cerebral cortex and liver slices respectively.

Deyrup (1953a) has noted that tissue slices show very little change in water content when immersed in sucrose solutions approximately isotonic with extracellular fluid. Table 4, *b - a*, confirms this

Table 3. *Changes in the sodium, chloride, potassium and water contents of rat cerebral cortex and liver on incubation at 0°*

Slices of tissue (0.1–0.2 g.) incubated 30 min. at 0° in 3.0 ml. of 160 m-equiv. NaCl and 5 m-equiv. KCl per l. Mean values $\pm$ s.e.

	No. of samples	Dry wt. (%)	Water/kg. dry solids (kg.)	m-equiv. per kg. dry solids		
				Na	Cl	K
Rat cerebral cortex						
(a) Before incubation	6	22.7 $\pm$ 0.3	3.40 $\pm$ 0.03	312 $\pm$ 19	219 $\pm$ 7	451 $\pm$ 17
(b) After incubation	6	17.5 $\pm$ 0.2	4.72 $\pm$ 0.04	788 $\pm$ 23	516 $\pm$ 13	194 $\pm$ 9
<i>b - a</i>	—	—	+1.32 $\pm$ 0.05	+476 $\pm$ 30	+297 $\pm$ 15	-257 $\pm$ 19
Rat liver						
(c) Before incubation	6	30.5 $\pm$ 0.6	2.28 $\pm$ 0.03	114 $\pm$ 14	121 $\pm$ 4	345 $\pm$ 6
(d) After incubation	6	23.1 $\pm$ 0.03	3.33 $\pm$ 0.03	477 $\pm$ 34	364 $\pm$ 20	167 $\pm$ 12
<i>d - c</i>	—	—	+1.05 $\pm$ 0.04	+363 $\pm$ 37	+243 $\pm$ 20	-178 $\pm$ 13

Table 4. *Changes in sodium chloride, potassium and water contents of guinea-pig kidney cortex slices on incubation in sucrose-containing medium*

Slices of tissue (0.1–0.2 g.) incubated 30–180 min. in 0.25 M sucrose at 0°. Mean values $\pm$ s.e.

	No. of samples	Dry wt. (%)	Water/kg. dry solids (kg.)	m-equiv. per kg. dry solids		
				Na	Cl	K
(a) Before incubation	14	23.7 $\pm$ 0.35	3.22 $\pm$ 0.04	350 $\pm$ 16	272 $\pm$ 7	365 $\pm$ 8
(b) In 0.25 M sucrose	4	23.5 $\pm$ 0.73	3.25 $\pm$ 0.08	155 $\pm$ 18	46 $\pm$ 16	179 $\pm$ 4
<i>b - a</i>	—	—	+0.03 $\pm$ 0.09	-195 $\pm$ 24	-226 $\pm$ 17	-186 $\pm$ 9

observation; the mean dry weight equals that of the control. However, measurement revealed marked losses of sodium, potassium and chloride from the tissue. These large losses of osmotically active tissue constituents indicate that though the tissue may be in osmotic equilibrium with its medium after incubation in such a way as to preserve the normal state of tissue hydration, this finding bears little relationship to the initial osmotic state of the tissue.

DISCUSSION

In 1951 Mudge (1951*b*) presented results from studies *in vitro* showing that the swelling produced by a variety of inhibitors of metabolism was associated with an uptake of ions as well as of water by the swollen tissue. The present results are fully in accord with his conclusion that: 'Depression of metabolic activity is, therefore, associated with an isosmotic increase in cellular hydration.'

The gain in tissue water and solute in the present experiments is probably shared by both the intra- and extra-cellular phases of the tissue. Thus, Robinson (1950) found by measurement of the inulin and sucrose spaces in slices of rat renal cortex that the 'extracellular' water remains an approximately constant fraction (26%) of the weight of the slices whether tissue hydration is normal or increased. This finding has been confirmed with slices of guinea-pig cortex incubated aerobically at 0° (Whittam, 1954).

Several workers have noted a loss of tissue solids during incubation *in vitro* (Robinson, 1949;

Aebi, 1952*a*). This may amount to 8–18% of the nitrogen content of liver slices (Aebi, 1952*a*). Others have demonstrated, however, that re-incubation of swollen tissue in media favourable to metabolic processes results in a return of tissue hydration and electrolyte composition toward control levels (Turner, Eggleston & Krebs, 1950; Krebs, Eggleston & Turner, 1951; Robinson, 1950; Mudge, 1951*a*; Deyrup, 1953*b*; Whittam & Davies, 1953). Hence the loss of tissue solids during incubation does not appear to affect the present results.

The finding that tissues gain solute as well as water invalidates the argument that the process of swelling involves a movement only of water into cells in response to a high intracellular osmotic pressure. Another explanation to account for changes in tissue hydration is required.

Cell	Medium
A^{n-}	Na_o^{+}
K_i^{+}	Cl_o^{-}
Cl_i^{-}	K_o^{+}

Fig. 1.

As shown in Fig. 1, a cell and its medium may be considered to constitute a 'double' Donnan system in which the cell membrane separates a non-diffusible intracellular anion, A^{n-} with n negative charges, and an extracellular cation, Na^{+} , excluded from the cell. The osmotic effects of these two ions will thus counterbalance each other, and the

normal cell volume will be maintained. The extra-cellular position of Na^+ is, however, dependent upon a supply of metabolic energy. When metabolism is disturbed Na^+ enters the cells. It will be shown that this must result, as well, in an entry of extracellular anions into the cell, a rise of intracellular osmotic pressure and swelling of the cell.

The distribution of diffusible ions is described by:

$$[\text{K}^+]_i [\text{Cl}^-]_i = [\text{K}^+]_o [\text{Cl}^-]_o, \quad (1)$$

and
$$[\text{K}^+]_i = [\text{Cl}^-]_i + n[\text{A}^{n-}]. \quad (2)$$

Then
$$([\text{Cl}^-]_i + n[\text{A}^{n-}]) [\text{Cl}^-]_i = [\text{K}^+]_o [\text{Cl}^-]_o, \quad (3)$$

or
$$[\text{Cl}^-]^2 + n[\text{A}^{n-}] [\text{Cl}^-]_i = [\text{K}^+]_o [\text{Cl}^-]_o,$$

where $[]$ indicate concentrations, activity coefficients being assumed to be unity throughout, and $_i$ and $_o$ refer to intra- and extra-cellular concentrations respectively.

If metabolism is disturbed so that Na^+ which diffuses into the cell is no longer actively extruded, the distribution of diffusible ions becomes:

$$\frac{[\text{K}^+]_i}{[\text{K}^+]_o} = \frac{[\text{Na}^+]_i}{[\text{Na}^+]_o} = \frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i}, \quad (4)$$

and
$$[\text{K}^+]_i + [\text{Na}^+]_i = [\text{Cl}^-]_i + n[\text{A}^{n-}]. \quad (5)$$

Substituting into (5) the values of $[\text{K}^+]_i$ and $[\text{Na}^+]_i$ obtained from (4)

$$[\text{Cl}^-]_i^2 + n[\text{A}^{n-}] [\text{Cl}^-]_i = [\text{K}^+]_o [\text{Cl}^-]_o + [\text{Na}^+]_o [\text{Cl}^-]_o. \quad (6)$$

The left-hand terms of equations (3) and (6) contain the variable, $[\text{Cl}^-]_i$, of interest, and a constant, $n[\text{A}^{n-}]$. The right-hand terms can all be considered constants if the same medium is used because of the relatively large volume of medium. A comparison of equations (3) and (6) indicates that the value of $[\text{Cl}^-]_i$ must be greater in the latter.

The osmotic pressure difference, ΔP , between cell and medium in this simple example may be expressed:

$$\Delta P = RT (\Sigma \text{intracellular ions} - \Sigma \text{extracellular ions})$$

assuming osmotic coefficients of unity for each ion species in the hypothetical system. As derived in Appendix I, with sodium excluded from the cell,

$$\Delta P_1 = RT \{ [\text{A}^{n-}] + \sqrt{n^2 [\text{A}^{n-}]^2 + 4[\text{K}^+]_o [\text{Cl}^-]_o} - 2[\text{Cl}^-]_o \} \quad (7);$$

whereas with sodium freely entering the cell,

$$\Delta P_2 = RT \{ [\text{A}^{n-}] + \sqrt{n^2 [\text{A}^{n-}]^2 + 4([\text{K}^+]_o [\text{Cl}^-]_o + [\text{Na}^+]_o [\text{Cl}^-]_o)} - 2[\text{Cl}^-]_o \}. \quad (8)$$

Inspection of equations (7) and (8), in which all terms are considered constants when the external medium is the same, indicates that $\Delta P_2 > \Delta P_1$. This increase in ΔP_2 , which is a necessary consequence of the inability of the cells to extrude sodium actively when metabolic processes are inhibited, will result in a swelling or increased hydration of the cell if its membrane is distensible.

Re-establishment of normal metabolism, by causing extrusion of sodium from cells, will secondarily reduce the tissue swelling. Such a mechanism is in accord with the recently proposed hypothesis of Wilson (1954).

For purposes of the present argument it is immaterial whether the cell membrane is assumed impermeable to sodium or whether the membrane is permeable, but the accumulation of sodium in the cell is actually prevented by active extrusion of sodium from the cells. Abundant evidence (see Ussing, 1952; Steinbach, 1952; Hodgkin & Keynes, 1955) supports the latter alternative. The prevention of sodium accumulation as a consequence of an active uptake of potassium or of its selective intracellular accumulation (Ling, 1952, 1955) would likewise be compatible with the present concept. The quantity of sodium apparently normally present in the cell can be ignored for purposes of simplicity.

This explanation of the observed entry of chloride into tissues whose metabolism has been disturbed has been based on the currently accepted view that cations are the actively transported ions. Should it be shown alternatively that chloride is actively extruded from cells its accumulation in tissues would be a direct consequence of a disturbed metabolism.

The present hypothesis is intended to afford only a qualitative description of events as they actually occur in tissues. Much more information about compartment sizes, distribution of ions and ionic activities in tissues will be necessary before any quantitatively satisfactory formulation of this problem can be achieved. Also, if the osmotic pressure of the medium in which the tissue is incubated is produced by solute (e.g. by monosaccharides) other than the customary ions, the tissue swelling will then obviously depend on the degree to which this solute penetrates the cells.

In 1907 Lillie demonstrated that the osmotic pressure exerted by a given concentration of protein decreases with increasing concentration of neutral salts in the medium. It is theoretically possible therefore (see Appendix II) that swelling of tissues may be prevented, even when metabolism is inhibited, by placing them in hypertonic salt solutions. This is exactly what Opie (1949), Robinson (1950) and Aebi (1953) have found experimentally. From the above development it is apparent, however, that this finding does not justify the claim that the high concentrations of such media represent the true total solute concentration within metabolizing cells.

The present results are compatible with the view that the increase in tissue sodium content which has been repeatedly observed under conditions of impaired cellular metabolism is divisible into at

least two components, an ion exchange of extracellular sodium with intracellular potassium and a net movement of sodium into cells together with chloride. The much larger magnitude of the increase in tissue sodium content than of the reciprocal decrease in tissue potassium is thus accounted for. This large discrepancy between sodium gained and potassium lost is easily overlooked if the changes in tissue composition are expressed per unit weight of wet tissue rather than per unit weight of tissue solids. Thus Mudge (1951*a*) found a loss of potassium of 123 and 195 m-equiv. compared with a gain of sodium of 292 and 291 m-equiv./kg. tissue solids in his initial non-leached and leached slices of rabbit kidney cortex, respectively, as compared with the fresh tissue control. In spite of the large discrepancies between quantities of sodium and potassium gained and lost by the swollen tissue it was noted that, when expressed per kg. of wet tissue: 'Within the limits of the experimental error the changes in tissue Na were the reciprocal of K and the sum of Na and K remained constant.' Whittam & Davies (1953) likewise present figures showing reciprocal and approximately equal changes in wet tissue sodium and potassium content, although the recalculation of their data in Table 2 of this paper clearly reveals the much larger increase of sodium than loss of potassium in their study. It is apparent that if expressed on a wet tissue (or tissue water) basis, a simple addition or subtraction of an isotonic solution of a sodium salt to a tissue will produce reciprocal changes in tissue potassium without any potassium necessarily being lost or gained by the tissue.

from the medium, largely sodium chloride, to return the cells to their pre-incubation level. As only swelling in excess of the pre-incubation state of hydration has been considered in the calculations, it would be expected that the chloride uptake by the swollen tissues would represent more than simply an isotonic increase of chloride in the increment of water gained. In this respect, the quantity of anions lost in accompaniment with potassium from the cells during incubation can be calculated from Table 3, in which experiments chloride was the sole anion of the medium. As stated in Results, this loss of intracellular anion per kg. of dry solids of cerebral cortex and liver was 78 and 58 m-equiv. respectively. The corresponding excess of chloride above an isotonic entry was 80, i.e. $(225 - 165) \times 1.32$, and 69, i.e. $(231 - 165) \times 1.05$, m-equiv. respectively. The correspondence of these values is sufficiently close, if the anions lost were univalent, to lend support to this argument.

It has been frequently stated that the sum of intracellular sodium and potassium concentrations in mammalian tissues considerably exceeds their combined concentrations in the medium or extracellular fluid (Elkinton, Winkler & Danowski, 1944; Whittam & Davies, 1953; Bartley *et al.* 1954). Though this finding is still cited occasionally as evidence for a higher intracellular than extracellular osmotic pressure, the realization is increasing that this may simply be a necessary requirement to maintain osmotic equality between the two fluids as a consequence of a considerable quantity of polyvalent anion of high molecular weight within the cells (Mudge, 1953). Assuming

Table 5. *Comparison of the calculated chloride concentration in the increment of water gained during tissue swelling with the estimated chloride concentration of the medium*

Data from Tables 1-4.

Tissue	No. of comparisons	Calc. Cl concn. in water (m-equiv./l.)	Cl concn. of medium (m-equiv./l.)	Difference (m-equiv./l.)
(1) Kidney cortex (Table 1)	14	154	135	19
(Table 2)	—	166	135	31
(2) Cerebral cortex	6	225	165	60
(3) Liver	6	231	165	66

As noted in Results and Table 5, the calculated chloride concentration in the increment of water gained by the swollen tissues was greater than the concentration of chloride of the medium. This finding is probably a necessary consequence of the manner in which the control data were obtained. Thus unincubated fresh tissue slices were compared with slices placed in media into which they might be expected to lose solute. Any loss of intracellular, osmotically active solute from the cells during incubation would have to be replaced by solute

an extracellular volume of 26 % of the wet weight of the tissue (Robinson, 1950), the values of Table 1 can be used to calculate an 'intracellular' concentration of sodium plus potassium before incubation of 256 m-equiv. which decreased to 207 m-equiv./l. of intracellular water after metabolism was inhibited although the sodium plus potassium concentration of the medium remained constant at approximately 160 m-equiv./l. This decline in the gradient of intracellular to extracellular cation concentrations need not signify a fall in

intracellular total solute concentration consequent upon inhibition of metabolic processes. As Mudge pointed out (1951*b*) such a drop would be expected from the entrance of univalent anions into the cell (see Appendix III).

Stern *et al.* (1949) were the first to observe the dependence of tissue hydration on cellular respiration. Their observation has been repeatedly interpreted since as evidence for an active transport of water by cells. Without proving or disproving the possibility that primary active transport of water is a common functional property of cells (mammalian renal tubular cells, at least, would seem to be capable of this task) the present paper emphasizes that the changes in hydration which may be observed on incubation *in vitro* can be satisfactorily explained as a passive movement of water accompanying shifts of ions whose distribution in tissues is dependent upon a source of metabolic energy.

In the absence of a rigid cell membrane a water pump would be necessary to maintain within cells a total solute concentration of two to three times the concentration of the medium. However, such a high intracellular concentration should result in the entry of a fluid hypotonic to the medium in order that the water activity may become uniform throughout the tissue fluid when energy metabolism is inhibited. The fact that the fluid which entered the tissues during swelling was not hypotonic to the medium would seem to exclude the presence of such high intracellular concentrations. Although the presence of small differences in concentration cannot be excluded by the present study, the results are most simply explained on the assumption that osmotic equality exists between intra- and extra-cellular fluids of metabolizing tissues (or more correctly is higher within the cells only by the small pressure exerted by the membrane tension).

SUMMARY

1. The swelling of tissues *in vitro* which occurs when energy metabolism is disturbed was studied. Changes in water, sodium, chloride and potassium

contents of slices of guinea-pig kidney cortex, rat liver and rat cerebral cortex were determined.

2. The fluid entering the tissues in the process of swelling was not water alone, as some previous workers have tacitly assumed, but a solution approximately isotonic with the medium. The quantity of sodium entering the tissue was accounted for by that which exchanged on an ionic basis with intracellular potassium plus that which entered together with chloride.

3. This finding invalidates the argument that tissue swelling is a movement of water alone attracted by a high intracellular osmotic pressure.

4. The dependence of fluid exchange of tissues on tissue metabolism can be explained by the requirement of metabolic energy for the maintenance of ionic gradients in tissues. Active extrusion of sodium from cells maintains a higher extracellular than intracellular concentration of this ion, which counterbalances the osmotic effect of intracellular non-diffusible colloid, thus preserving the normal cellular volume. Accumulation of sodium in cells when their metabolism is inhibited has as a consequence an increase of tissue chloride. The temporary rise in osmotic pressure within cells produced by such entry of ions of the medium manifests itself as an increase in tissue hydration. Re-establishing metabolic activity in the tissue will result in extrusion of sodium and chloride with reduction in tissue hydration.

5. The prevention of tissue swelling in hypertonic saline solutions, even when tissue metabolism is depressed, may also be explained on this basis.

6. The results are most simply explained on the assumption that osmotic equality exists between intra- and extra-cellular fluids of metabolizing tissues.

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APPENDIX

I. Derivation of ΔP for the simplified system illustrated in Fig. 1

Let ΔP equal the osmotic pressure difference between cell and medium. Then (Hitchcock, 1946) for the simple system illustrated in Fig. 1,

$\Delta P = RT (\Sigma \text{intracellular ions} - \Sigma \text{extracellular ions})$ when molal concentrations of ions are summed and unit osmotic activity coefficients are assumed for each ion species.

With Na excluded from the cell,

$$\Delta P_1 = RT \{ ([K^+]_i + [Cl^-]_i + [A^-]_i) - ([Na^+]_o + [K^+]_o + [Cl^-]_o) \}.$$

Substituting values for $[K^+]_i$ and $[Cl^-]_i$ obtained from equations (2) and (3) of the text, this expression becomes

$$\Delta P_1 = RT \{ [A^-]_i + \sqrt{\{n^2[A^-]_i^2 + 4([K^+]_o [Cl^-]_o)\}} - 2[Cl^-]_o \}. \quad (7)$$

With Na^+ freely entering the cell:

$$\Delta P_2 = RT \{ ([\text{K}^+]_i + [\text{Na}^+]_i + [\text{Cl}^-]_i + [\text{A}^{n-}]) - ([\text{Na}^+]_o + [\text{K}^+]_o + [\text{Cl}^-]_o) \}.$$

Substituting values for $[\text{K}^+]_i + [\text{Na}^+]_i$ and for $[\text{Cl}^-]_i$ obtained from equations (5) and (6) of the text, this becomes

$$\Delta P_2 = RT \{ [\text{A}^{n-}] + \sqrt{n^2 [\text{A}^{n-}]^2 + 4([\text{K}^+]_o [\text{Cl}^-]_o + [\text{Na}^+]_o [\text{Cl}^-]_o)} - 2[\text{Cl}^-]_o \}, \quad (8)$$

in which ΔP is expressed as a function of concentration and charge of intracellular non-diffusible anion, $[\text{A}^{n-}]$, and the concentrations of ions in the medium.

II. Effect on ΔP_2 of varying the solute concentration in the medium when Na^+ is free to enter the cell

Again, as $[\text{Na}^+]_o + [\text{K}^+]_o = [\text{Cl}^-]_o$, the total ionic concentration of the medium may be represented by $2[\text{Cl}^-]_o$ and equation (8) rewritten as

$$\frac{\Delta P_2}{RT} - [\text{A}^{n-}] = \sqrt{n^2 [\text{A}^{n-}]^2 + 4[\text{Cl}^-]_o^2} - 2[\text{Cl}^-]_o. \quad (9)$$

As $[\text{A}^{n-}]$ is assumed to remain constant the effect of altering the medium concentration, $2[\text{Cl}^-]_o$, on the right-hand portion of equation (9) will be considered.

Fig. 2. Calculated values for the function $\Delta P_2/RT - [\text{A}^{n-}]$ from equation (9) of Appendix II which show the expected decrease of ΔP_2 (the difference in osmotic pressure between cell and medium when metabolism is inhibited) with increase of concentration of the medium. Units on both abscissa and ordinate are expressed as factors of $n[\text{A}^{n-}]$.

If $2[\text{Cl}^-]_o$ is much smaller than $n[\text{A}^{n-}]$ the right-hand expression has a maximum value of $n[\text{A}^{n-}]$. If $2[\text{Cl}^-]_o$ is much larger than $n[\text{A}^{n-}]$ the right-hand expression has a minimum value of 0. With $n[\text{A}^{n-}]$ kept constant this latter state can be approximated by increasing the concentration of medium, $2[\text{Cl}^-]_o$, and would have the effect of reducing the value of ΔP_2 to a value as low as any value for ΔP_1 that might exist. Fig. 2 shows a

graph of calculated changes in $\Delta P_2/RT - [\text{A}^{n-}]$ with increase of medium concentration. Though the present results are inadequate to permit an accurate assessment of an actual value for $n[\text{A}^{n-}]$ it is likely that changes in the curve between concentrations of the medium of approximately 1 to 4 in Fig. 2 are pertinent to the present discussion.

III. Decrease in sum of intracellular cation concentrations subsequent to entry of chloride into cells

Referring again to the simplified model in Fig. 1 and letting subscripts 1 and 2 denote the intracellular ions before and after entry of Na^+ into the cell, we have

$$\Delta P_1 = RT \{ ([\text{K}^+]_1 + [\text{Cl}^-]_1 + [\text{A}^{n-}]_1 - ([\text{Na}^+]_o + [\text{Cl}^-]_o + [\text{K}^+]_o)) \},$$

$$\Delta P_2 = RT \{ ([\text{K}^+]_2 + [\text{Na}^+]_2 + [\text{Cl}^-]_2 + [\text{A}^{n-}]_2 - ([\text{Na}^+]_o + [\text{Cl}^-]_o + [\text{K}^+]_o)) \},$$

in which all ion symbols refer to concentrations.

Substituting for $[\text{Cl}^-]_1$ its value from equation (2) of text; ΔP_1 may be rewritten

$$\frac{\Delta P_1}{RT} = 2[\text{K}^+]_1 - n[\text{A}^{n-}]_1 + [\text{A}^{n-}]_1 - ([\text{Na}^+]_o + [\text{Cl}^-]_o + [\text{K}^+]_o). \quad (a)$$

Similarly, using equation (5) of the text, the expression for ΔP_2 becomes,

$$\frac{\Delta P_2}{RT} = 2[\text{K}^+]_2 + 2[\text{Na}^+]_2 - n[\text{A}^{n-}]_2 + [\text{A}^{n-}]_2 - ([\text{Na}^+]_o + [\text{Cl}^-]_o + [\text{K}^+]_o). \quad (b)$$

Subtracting (a) from (b)

$$(2[\text{K}^+]_2 + 2[\text{Na}^+]_2 - (n-1)[\text{A}^{n-}]_2) - (2[\text{K}^+]_1 - (n-1)[\text{A}^{n-}]_1) = \frac{\Delta P_2 - \Delta P_1}{RT}. \quad (c)$$

If the difference in osmotic pressures $\Delta P_2 - \Delta P_1$ is minimized by swelling of the cells, the right-hand expression in (c) approaches a limiting value of zero and (c) may be approximated by

$$2[\text{K}^+]_1 - (n-1)[\text{A}^{n-}]_1 = 2[\text{K}^+]_2 + 2[\text{Na}^+]_2 - (n-1)[\text{A}^{n-}]_2. \quad (d)$$

But the increase of fluid in the cells associated with tissue swelling will reduce the concentration of the fixed quantity of intracellular polyvalent anion so that

$$(n-1)[\text{A}^{n-}]_1 > (n-1)[\text{A}^{n-}]_2.$$

The effect of this inequality on equation (d) is to make

$$[\text{K}^+]_1 > [\text{K}^+]_2 + [\text{Na}^+]_2,$$

or the sum of the concentrations of intracellular cations decreases subsequent to entry of chloride into the cells.

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Ascorbic Acid and Glutathione as Respiratory Carriers in the Respiration of Pea Seedlings

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Szent-Györgyi (1931), from a knowledge of the behaviour of reduced glutathione (GSH) and ascorbic acid (AA), first postulated a scheme whereby these substances might act as respiratory carriers. The subsequent discovery of the enzymes dehydroascorbic acid reductase (Crook & Hopkins, 1938; Crook, 1941) and glutathione reductase (Mapson & Goddard, 1951; Conn & Vennesland, 1951) made it possible to visualize a system whereby hydrogen may be transferred from substrates such as isocitrate and malate to molecular oxygen. Mapson & Goddard (1951), in fact, were able to demonstrate the reduction of dehydroascorbic acid (DHA) as a result of hydrogen transfer from either malate or isocitrate via triphosphopyridine nucleotide (TPN) and GSH. Such a system might be expected to consume oxygen if, in addition to these enzymes, a terminal oxidase capable of oxidizing

ascorbic acid was present. The reactions concerned may be formulated as follows:

where SH_2 represents an oxidizable substrate.

The work described here aimed to demonstrate the presence of such a system in extracts *in vitro*, and to evaluate its importance in the respiration of the tissue *in vivo*.

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