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Regulation of Intracellular Fluid Volume and Disease

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Over the past few decades many studies have been made on the factors regulating the volume and composition of the extracellular fluids. Through the efforts primarily of Darrow [1] in the 1940's the significance in disease of disturbances of intracellular fluid composition, particularly of potassium deficiency and excess, is also appreciated. However, disturbances in regulation of the volume of the intracellular fluids have received little attention. Only in the case of brain swelling, with all the associated problems of increased intracranial pressure, does disturbance of intracellular volume come to the attention of the physician. The subject has been ignored, not willfully, but because of the difficulties in recognizing its presence. Simple clinical parameters, such as can be used to monitor deficiency or excesses of extracellular fluid volume, are not available to provide equivalent information about the volume of the intracellular fluids. Even with technics of considerable physiologic sophistication, at best only indirect estimates of intracellular fluid volume can be made in the whole animal or isolated tissue, technics too cumbersome and insensitive to be of clinical value. I would like to call attention to the possible occurrence of disturbances in intracellular fluid volume and the role of such changes in the pathogenesis of important clinical disease.

The regulation of intracellular fluid volume has been understood, at least in principle, for several years [2–4]. Unlike the situation that exists for the water content of the extracellular fluids, in which a single organ, the kidney, has the primary role in volume control, regulation of the two thirds of total body water which comprise the intracellular fluids is a function of each individual cell. This assertion requires modification to the extent that neighboring cells are coupled electrically [5] and may function with respect to volume regulation as a true syncytium.

It is well known that the cell has a high protein content, a significant portion of which exists in a soluble state in which it may be expected to exert an oncotic pressure. The interstitial fluid bathing the cells, however, is relatively low in its content of colloid. From studies with isotopically labeled tracers it is now established that the cell membrane is permeable to water and to all the small solute molecules which contribute significantly to the osmolality of the extracellular fluids; these solutes are largely the salts of sodium. One might expect that the oncotic pressure exerted by the intracellular proteins

TABLE I Changes in the Sodium, Potassium, Chloride and Water Contents of Guinea Pig Kidney Cortex Slices on Incubation at 0°C

Data	No. of Samples	Dry Weight (%)	Water/kg Dry Solids (kg)	mEq/kg Dry Solids		
				Sodium	Chloride	Potassium
(a) Before incubation	14	23.7 ± 0.35	3.22 ± 0.04	350 ± 16	272 ± 7	365 ± 8
(b) After incubation	14	19.4 ± 0.29	4.16 ± 0.05	579 ± 18	417 ± 17	240 ± 7
b − a	. . .	. . .	+0.94 ± 0.06	+229 ± 24	+145 ± 18	−125 ± 11

NOTE: Slices of tissues (0.1 to 0.2 gm) incubated 30 to 50 minutes in 2.0 ml of bicarbonate-saline solution, pH 7.4, gas phase O_2 + CO_2 (95:5). Mean values ± standard error.

would cause disastrous swelling by drawing fluid from the extracellular compartment into the cells. Such swelling obviously does not occur in healthy tissues in vivo. One is therefore justified in inquiring into the nature of the force that normally counteracts this tendency toward swelling of cells and acts, thereby, to preserve the normal intracellular fluid volume.

The maintenance of normal intracellular volume is an energy-requiring process. In 1949 Stern et al. [6] observed that the swelling of various tissues in vitro was dependent upon tissue respiration; under anaerobic conditions various tissues of the guinea pig showed large gains in weight, attributable to increased water content, which were minimized or prevented by incubation in aerobic conditions. This type of observation has been repeated by a number of other workers using various tissues and a variety of means of inhibiting metabolism [7–10]. In all instances inhibition of metabolism has been associated with increased water content of the tissue studied.

A much older observation which goes back at least to Sabbatani's work at the turn of this century [11–14] is that swelling of tissues in vitro can be prevented by incubating tissues in highly concentrated media rather than the usual isotonic saline media.

These two observations, that tissue swelling is dependent on tissue metabolism and that it can be prevented in hypertonic media, gave rise at first to the misconception that the water in intracellular fluids was at a lower chemical potential than in the extracellular fluids and that this gradient was maintained by energy metabolism. We know now that no such gradients exist in fact, except in tissues elaborating anisosmolar secretions. What earlier clinicians accepted intuitively regarding the equality of intracellular and extracellular osmolality has been established by appropriate measurements [15–17]. In fact, with the high permeability of most cell membranes to water, it is unlikely that sufficient energy is available from metabolism to maintain cell volume by pumping water out of the cells were significant gradients of water activity to exist between cell interior and exterior.

With the osmolality of intracellular fluids equal to that of extracellular fluid, the intracellular volume must be a function of the quantity of solute within the cell, water moving back and forth across cell membranes passively as necessary to equalize its activity in intracellular and extracellular fluids. Mudge [7] first demonstrated that solute movements accompanied the changes in tissue water associated with changes in tissue metabolism. Table I [3] shows the changes in tissue content of sodium, chloride, potassium and water which accompany the inhibition of metabolism produced by incubation at 0°C of slices of guinea pig kidney cortex. The incubating medium was a Krebs-Ringer's solution and the period of incubation was thirty to fifty minutes. Control slices were unincubated, fresh slices of tissue. Results are expressed per kilogram of tissue solids. The tissue swelling is indicated by the increase in tissue water of 0.94 L. The increase in tissue sodium and loss of tissue potassium noted by others is seen. Of perhaps more importance to the present argument is the large gain in tissue chloride which occurred. One can calculate the concentration of chloride in the increment of water gained; 154 mEq/L. As the concentration of chloride in the incubating medium was 135 mEq/L, the data indicate that the gain in tissue water content in these experiments represented essentially an isosmolar entry of medium into the tissue. This finding invalidates the assumption that tissue swelling is due to only net movements of water between tissue and medium; the primacy of solute movement followed by water movement is confirmed. By reestablishing metabolism these changes are reversible [8–10,18–20].

Against this background we may formulate the present view of the volume regulation, as depicted in Figure 1. On the left is shown a metabolizing cell with normal volume. Its intracellular content of nondiffusible macromolecules, largely proteins and organic phosphates of net negative electrical charge, is indicated by A^{n-}. At least some of these nondiffusible molecules are in solution and exert an osmotic pressure tending to draw extracellular fluid into the cell. Figure 1 indicates that this tendency is offset by the obligatory extracellular position of the sodium ion, Na^+. The sodium ion is maintained in its largely extracellular position not by impermeability of cell membranes to sodium, as was thought to be the case prior to the advent of radioactive isotopes of sodium, but because sodium ions are largely excluded from cells as a result of active sodium extrusion mechanisms, "pumps," located in the outer plasma membranes of all cells, which continuously extrude sodium from the cell interior as rapidly as the sodium enters the cell by diffusion from high extracellular to low intracellular concentrations. Sodium, which is continuously falling "downhill" into the cell, is constantly being pumped out by active, energy-requiring transport mechanisms. The consequences

of such active extrusion of sodium * are (1) a membrane potential oriented with cell interior electronegative to cell exterior; (2) exclusion of small, negatively charged ions, like chloride (Cl^-), from cell interior by the membrane potential; (3) accumulation of potassium ions (K^+) within the cell. The high intracellular concentration of K^+ offsets the intracellular negativity so that K^+ is distributed close to its electrochemical equilibrium, between cell interior and exterior as a first approximation. Actually, it appears that most cells have an additional active accumulatory system for concentrating intracellular potassium [21–23].

The balance of solute distribution is such as to stabilize the cell volume; the extracellular position of sodium counter-balances the osmotic effect of intracellular colloids; a "double" Donnan system is established. The extracellular position of sodium is dependent, however, upon a continuous supply of metabolic energy for the extrusion of sodium from the cell as rapidly as it enters. A dynamic steady state rather than an equilibrium or static condition thus preserves cell volume. This can be readily seen by the consequences that follow inhibiting the supply of metabolic energy that is constantly required to maintain the sodium "pumps." The right hand portion of Figure 1 indicates schematically that the sodium which is continuously "falling downhill" into the cell can no longer be extruded. The accumulation of this positive ion within the cell reduces the cell membrane potential, allowing chloride ions to enter the cell and potassium ions to leave. However, the result is that more sodium and chloride must enter than potassium leaves the cell [3] (Table I) so that there is a net gain of solute within the cell. Water follows passively and the cell swells. As long as the supply of metabolic energy is made available to the sodium transport system while the cell is still alive, the process is reversible; sodium will again be pumped out and the steady state will be reestablished, with return of cell volume to normal.

It has been estimated that more than one third of the energy metabolism of resting muscle cells is expended to maintain this steady-state, extracellular position of sodium ions [24] and thus preserve the volume of the cell.

One might ask why in animal tissues so much energy must be spent simply to maintain the status quo? Animal cells are dependent upon a large surface to volume ratio to allow rapid exchanges of metabolites across cell membranes; large cells would of necessity mean sluggish animals. Furthermore, pliability and mobility of cells requires low membrane tensions. When such measurements of cell membrane tensions have been made, very low tensions have been found in animal cells. Some years ago E. N. Harvey [25] summarized this finding by stating, "We are accustomed to thinking in terms of air water surface tensions of 73 dynes/cm so that it is hard to realize that tensions

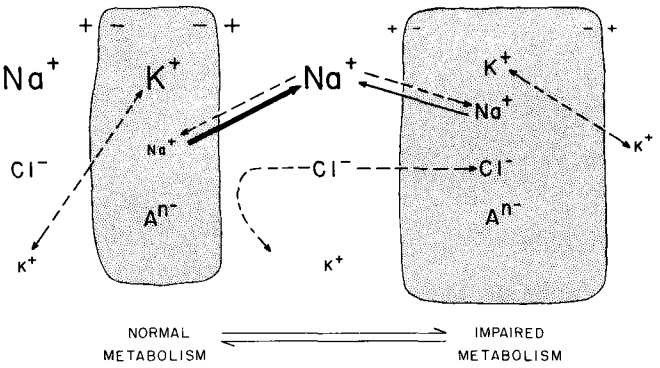

Fig. 1. Schematic representation of normal cell on left and the swollen cell on right resulting from temporary inhibition of energy metabolism. The changes are depicted as reversible.

of cells is at least some 1,000 times, perhaps 10,000 times less." These low membrane tensions are possible because cell swelling is avoided by the ion transport system, just discussed. The only feasible alternative adjustment to the cell to prevent the swelling that would result from its content of intracellular colloids would be to surround itself with a rigid casing capable of withstanding the high swelling pressure that is involved. This latter is, of course, the function provided by the cellulose casing around each cell of the sessile members of the vegetable kingdom.

The energy stored in these ion gradients between cells and their extracellular media has been adapted to provide nerve conduction in certain specialized cells, and contractility in others. Thus we owe our motility and consciousness to the ion transport mechanisms probably developed initially to prevent disastrous cell swelling in a saline environment.

This bit of physiology may be entertaining but what is its relevance to human disease? As one who firmly believes that with further understanding of physiologic process will come better comprehension of disease and improvement in our ability to prevent or treat it, this question has bothered me for some time. It has provided little satisfaction to realize that conventional histologic technics involving fixation, dehydration and embedding of tissues would so distort cell volume as to make major changes unrecognizable. But even with ideal technics that preserved true cell volumes, an increase of 50 per cent in volume would involve an increase in radius of individual cells of only 15 per cent. With cells cut at various distances from their equatorial plane in histologic section, who could recognize such changes even if all cells were perfect spheres?

Recently, however, a series of elegant studies have been published which suggest that cell swelling may have a very important bearing on major human disease. Ames and associates [26,27] have been investigating rabbit retina in vitro and have developed a preparation which remains viable in a simple medium as indicated by continued respiration and functionally by the discharge of nerve impulses in the attached optic nerve evoked by light impinging on the retina. Interest in how the function and survival of the retina were dependent upon its metabolism led to incubating the tissue

* This ion distribution may be more rigorously described in terms of Donnan distributions [3] rather than membrane potentials, but for descriptive purposes and to give some appreciation for membrane potentials this less rigorous approach followed in the text seems justified.

under anaerobic conditions. In the presence of adequate glucose in the incubation medium they found that the isolated retina preparation would survive for periods in excess of one hour; reoxygenation of the medium resulted in return of electrical discharge through the optic nerve in response to light flashes on the retina. Since the retina is known to possess as high a metabolic rate as any portion of the central nervous system [18], and since anoxia for only a few minutes in the intact animal leads to irreversible brain death [28], this prolonged survival of isolated retina in the complete absence of oxygen seemed to Ames to require explanation. His tentative conclusion, that obstruction of blood flow to the brain for only a few minutes must somehow interfere with the return of blood flow to the brain when the obstruction was released, proved correct. Injection of particulate carbon black into the carotid arteries upon termination of obstruction of these vessels revealed that little of the carbon particles reached the capillaries of considerable portions of the brain [29,30]. Careful histologic studies [31] have shown that the failure of circulation to return to the brain resulted from swelling of the perivascular glia cells to an extent which wholly or partially occluded the capillaries of the brain. Thus even transient ischemia interfered with the availability of metabolic energy to pump sodium out of these critically located cells. The resultant swelling from the uptake of extracellular fluid, largely plasma, obstructed the capillaries and also led to an increase in blood viscosity preventing return of blood to the brain, thus sustaining further ischemia, more cell swelling and finally tissue death from prolonged anoxia.

One cannot but wonder how often such a sequence of self-sustaining changes occurs in tissues, leading finally to death of the tissue [32–35]. Is the same vicious cycle involved in the development of strokes, whether from arteriosclerosis, cerebral hemorrhage or embolism? Does the ischemia of the myocardium which develops during exertion in people with atherosclerotic coronary vessels interfere with sodium transport and initiate the vicious cycle of cell swelling, vascular obstruction, sustained ischemia with eventual death? Does acute tubular necrosis and renal failure follow transient renal ischemia because of the same sequence of events? Might therapy be effectively directed toward measures which prevent or reverse cell swelling [36,37]? Is this perhaps the means by which mannitol infusions have seemed to be helpful in the early stages of acute renal failure?

At the present time one can only ask the questions; further work will be required to provide answers. However, a direction for investigations may be indicated. It seems possible that disturbances in cell volume in disease may not be recognizable in generalized systemic changes but may have been masquerading under our noses as an important factor in such major human diseases as strokes, myocardial infarctions and acute renal failure.

REFERENCES

1. Darrow DC: The retention of electrolyte during recovery from severe dehydration due to diarrhea. J Pediat 28: 515, 1946.
2. Wilson TH: Ionic permeability and osmotic swelling of cells. Science 120: 104, 1954.
3. Leaf A: On the mechanism of fluid exchange of tissues in vitro. Biochem J 62: 241, 1956.
4. Leaf A: Maintenance of concentration gradients and regulation of cell volume. Ann NY Acad Sci 72: 396, 1959.
5. Lowenstein WR: Permeability of membrane junctions. Conference on biological membranes: recent progress. Ann NY Acad Sci 137: 441, 1966.
6. Stern JR, Eggleston LV, Hems R, Krebs HA: Accumulation of glutamic acid in isolated brain tissue. Biochem J 44: 410, 1949.
7. Mudge GH: Electrolyte-water metabolism of rabbit kidney slices: effect of metabolic inhibitors. Amer J Physiol 167: 206, 1951.
8. Robinson JR: Osmoregulation in surviving slices from the kidneys of adult rats. Proc Roy Soc (Biol) B137: 378, 1950.
9. Deyrup I: Reversal of fluid uptake by rat kidney slices immersed in isosmotic solutions in vitro. Amer J Physiol 175: 349, 1953.
10. Whittam R, Davies RE: Active transport of water, sodium, potassium and α-oxoglutarate by kidney-cortex slices. Biochem J 55: 880, 1953.
11. Sabbatani L: Détermination du point de congélation des organes animaux. J Physiol 3: 939, 1901.
12. Gömöri P, Molnár S: Die Störung der Osmoregulation der Gewebe bei der Wasservergiftung. Arch exp Path Pharmakol 167: 459, 1932.
13. Opie EL: The movement of water in tissues removed from the body and its relation to movement of water during life. J Exp Med 89: 185, 1949.
14. Aebi H: Elektrolyt-akkumulierung and Osmoregulation in Gewebsschnitten. Helv Physiol Pharmacol Acta 11: 96, 1953.
15. Leaf A, Chatillon JY, Wrong O, Tuttle EP, Jr: The mechanism of the osmotic adjustment of the body cells as determined *in vivo* by the volume of distribution of a large water load. J Clin Invest 33: 1261, 1954.
16. Maffly RH, Leaf A: The potential of water in mammalian tissues. J Gen Physiol 42: 1257, 1959.
17. Appleboom JWTh, Brodsky WA, Tuttle WS, Diamond I: The freezing point depression of mammalian tissues after sudden heating in boiling distilled water. J Gen Physiol 41: 1153, 1958.
18. Terner C, Eggleston LV, Krebs HA: The role of glutamic acid in the transport of potassium in brain and retina. Biochem J 47: 139, 1950.
19. Krebs HA, Eggleston LV, Terner C: *In vitro* measurements of the turnover rate of potassium in brain and retina. Biochem J 48: 537, 1951.
20. Mudge GH: Studies on potassium accumulation by rabbit kidney slices: effect of metabolic activity. Amer J Physiol 165: 113, 1951.
21. Mudge GH: Electrolyte metabolism of rabbit-kidney slices: studies with radioactive potassium and sodium. Amer J Physiol 173: 511, 1953.
22. Shanes AM: Factors governing ion transfer in nerve. Electrolytes in Biological Systems (Shanes AM, ed), Washington, DC, American Physiological Society, 1955, p 157.

23. Relman AS: The physiological behavior of rubidium and cesium in relation to that of potassium. Yale J Biol Med 29: 248, 1956.
24. Levi H, Ussing HH: The exchange of sodium and chloride ions across the fibre membrane of the isolated frog sartorius. Acta Physiol Scand 16: 232, 1948.
25. Harvey EN: Tension at the cell surface. Protoplasmatologia 2: E5, 1954.
26. Ames A, III, Gurian BS: Effects of glucose and oxygen deprivation on function of isolated mammalian retina. J Neurophysiol 26: 617, 1963.
27. Webster H deF, Ames A, III: Reversible and irreversible changes in the fine structure of nervous tissue during oxygen and glucose deprivation. J Cell Biol 26: 885, 1965.
28. Wright RL, Ames A, III: Measurement of maximal permissible cerebral ischemia and a study of its pharmacologic prolongation. J Neurosurg 21: 567, 1964.
29. Kowada M, Ames A, III, Majno G, Wright RL: Cerebral ischemia. I. An improved experimental method for study; cardiovascular effects and demonstration of an early vascular lesion in the rabbit. J Neurosurg 28: 150, 1968.
30. Ames A, III, Wright RL, Kowada M, Thurston JM, Majno G: Cerebral ischemia. II. The no-reflow phenomenon. Amer J Path 52: 437, 1968.
31. Chiang J, Kowada M, Ames A, III, Wright RL, Majno G: Cerebral ischemia. III. Vascular changes. Amer J Path 52: 455, 1968.
32. Harman JW: The significance of local vascular phenomena in the production of ischemic necrosis in skeletal muscle. Amer J Path 24: 625, 1948.
33. Sheehan HL, Davis JC: Renal ischemia with failed reflow. J Path Bact 78: 105, 1959.
34. Kovacs K, Carroll R, Tapp E: Temporary ischemia of the adrenal gland. J Path Bact 91: 235, 1966.
35. Krug A, deRochamont W, Korb G: Blood supply of the myocardium after temporary coronary occlusion. Circ Res 19: 57, 1966.
36. Cantu RC, Ames A, III: Experimental prevention of cerebral vasculature obstruction produced by ischemia. J Neurosurg 30L50, 1969.
37. Strock PE, Majno G, Diethelm G: Protection of the vascular patency of ischemic dog limbs by various perfusate solutions, Organ Perfusion and Preservation (Norman J, ed), New York, Appleton, Century & Crofts, 1968, p 27.