A PROPOSED MECHANISM OF EXTRACELLULAR REGULATION OF MUSCLE COMPOSITION*

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The changes in muscle composition which occur in disturbances of extracellular electrolyte composition were systematically described by Darrow, *et al.*[8,11] and confirmed by others.[7,18] However, the mechanism responsible for these changes has not been satisfactorily elucidated. It is the purpose of this paper to describe briefly the composition of muscle in the major disturbances of acid-base balance and to present a general theory of the mechanism of electrolyte transfer between cell and extracellular fluid which could account for these findings. It is important to note that relatively little of the data to be discussed subsequently was obtained by the authors and their contribution is simply the interpretation of these data.

Muscle electrolyte composition is usually expressed in terms of the fat-free solids (FFS) rather than as concentration per liter of intracellular or extracellular fluid. Since the quantity of muscle solid per kilogram of animal is relatively constant and not affected by changes in extracellular volume, analysis of muscle expressed in these terms permits valid comparison of data obtained in various disturbances. The results of acute experiments are not given since time must elapse before marked alterations in muscle composition occur. The analyses presented are those of skeletal muscle of rats only. Analysis of human muscle obtained by biopsy suggests that changes in cell composition in man are similar.[16]

Metabolic alkalosis of the extracellular fluid produced either by administration of sodium bicarbonate or loss of chloride in excess of sodium leads to characteristic changes in muscle composition (Table 1).[7,8] The potassium content is low, averaging 38mM/100g.FFS and the intracellular sodium content is elevated, 7.7mM/100g.FFS. In addition, there is probably a rise in the concentration of undetermined anion (possibly lactate) in the serum as calculated from the difference between the concentrations of sodium and the sum of the concentrations of chloride and bicarbonate even when the quantity of undetermined anion is corrected for the change in base binding capacity of blood proteins with change in pH.

Metabolic acidosis of the extracellular fluid produced either by administration of HCl or NH_4Cl or loss of sodium in excess of chloride leads to

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** John and Mary R. Markle Scholar in Medical Science.

Received for publication July 23, 1952.

TABLE 1

BODY COMPOSITION IN DISTURBANCES OF ACID-BASE BALANCE

		Serum							*Muscle per 100 g. FFS*					
Group	*References*	Na mM/L	K mM/L	Cl mM/L	HCO_3 mM/L	*"Lactate"* ..	pH	pCO_2 mm.Hg.	H_2O g.	Cl mM	Na mM	K mM	P mM	Na_i* mM
Normals	8, 10, 7, 17, 18	141—146	4.1—5.4	99—102	21—25	..	7.35	45	334—346	5.1—7.2	9.4—10.6	46.1—48.9	32.0—34.0	3.1
Metabolic alk.	7	151	3.4	87	35	↑	7.44	53	314	3.9	11.9	37.8	30.4	7.7
Metabolic acid.	7	142	6.7	94	20	↓	7.28	44	337	4.5	6.1	49.8	33.2	1.6
Resp. acid.	5	143	4.8	85	41	↓	7.25	100	333	5.3	8.8	48.0	33.0	2.2
Resp. alk. (altitude)	10	143	5.6	124	↓	↑	↑	↓	342	9.1	12.0	45.0	32.5	3.5
K. def. alk.	6	145	2.7	93.0	30	?	7.50	38	334	5.7	18.5	34.1	34.2	11.9
Acute K excess (Group 8)	14	141	12.4	115	....	?	...	...	359	9.7	8.3	53.1	32.0	—2.3**

* Na_i—calculated as difference between total muscle sodium and extracellular sodium of muscle; concentration of extracellular sodium calculated from $[Na]_e = \frac{[Na]\,\text{serum}}{(H_2O)\,\text{serum}} \times .95$.

Volume of extracellular phase of muscle calculated from muscle chloride, assuming 1 mM of Cl/100 g. FFS is intracellular. Therefore, extracellular water of muscle $= \frac{(Cl)_m - 1}{[Cl]\ \text{serum}}{(H_2O)\,\text{serum} \times .95}$

** No significance in acute experiments since chloride probably enters cell in potassium poisoning.

changes in muscle composition opposite to those described in metabolic alkalosis[7, 11] (Table 1). The potassium content of muscle is elevated, 50mM/100g.FFS, and the intracellular sodium is low, 1.6mM/100g.FFS. There is probably a fall in concentration of undetermined anion in the serum.

Recently, muscle composition has been determined in rats made acidotic by exposure to an atmosphere containing 10-15 per cent carbon dioxide for 2-3 weeks[5] (Table 1). The average potassium content is 48mM/100g.FFS, approximating the upper limit of normal. The intracellular sodium is 2.2mM/100g.FFS, somewhat lower than normal. In addition, the undetermined anion concentration of serum is probably lowered even when corrected for pH change. These changes are very different from those obtained in metabolic alkalosis even though the serum bicarbonate concentration is elevated and the serum chloride concentration is lowered in both of these conditions.

Exposure to low atmospheric pressure probably produces chronic respiratory alkalosis by inducing overbreathing.[8] Analysis of rats exposed to such conditions revealed the following composition of muscle: potassium content, 45mM/100g.FFS, intracellular sodium, 3.5mM/100g.FFS[10] (Table 1). The potassium content is somewhat lower than normal and the sodium higher than normal. Furthermore, the serum lactate concentration has been shown to be elevated in respiratory alkalosis, and this elevation is not due simply to the activity of breathing since serum lactate fell to normal when 5 per cent CO_2 was substituted for room air.[8] These changes are different from those obtained in metabolic acidosis even though the extracellular bicarbonate concentration is depressed and the chloride concentration elevated in both conditions.

As can be seen from the four examples above, intracellular sodium does not universally vary directly with extracellular bicarbonate concentration or inversely with extracellular chloride concentration as previously considered[11] but rather with extracellular pH[5] (Fig. 1). Likewise, intracellular potassium varies inversely with extracellular pH. It is only in metabolic alkalosis and acidosis that cell composition varies with extracellular bicarbonate concentration since in these states $(pH)e$* and $(HCO_3)e$ vary in the same direction.

Excessive loss of potassium from the body usually leads to a rise in extracellular bicarbonate concentration. The muscle potassium is low in this condition (34mM/100g.FFS), and the intracellular sodium of muscle is elevated (11.9mM/100g.FFS).[6, 11] The increment of sodium amounts to approximately two-thirds of the decrement of potassium.[11, 17, 18] The difference (one-third) is considered to be hydrogen ion transferred from extra-

* Subscripts "e" and "i" refer to extracellular and intracellular phases respectively.

cellular fluid to cell to maintain electrical neutrality. The repair of alkalosis associated with potassium deficiency by means of potassium chloride administration does not require specific renal correction of the alkalosis and involves transfer of hydrogen ion from cell to extracellular fluid in exchange for potassium.[6]

Excess of potassium in the extracellular fluid can be maintained for only a short time in the presence of normal renal function, so that the converse of the above experiment cannot be performed readily. In acute experiments loads of potassium chloride produce a fall in extracellular bicarbonate con-

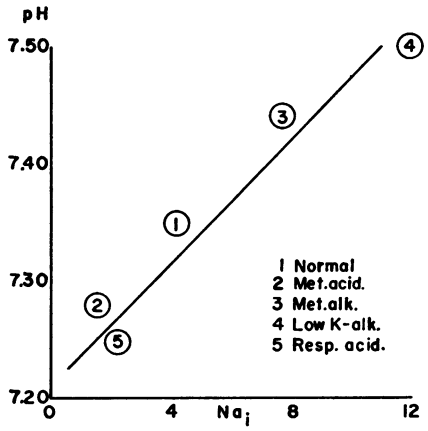

FIG. 1. Relationship of pH of extracellular fluid to intracellular sodium content of muscle expressed as mM/100 g. FFS.

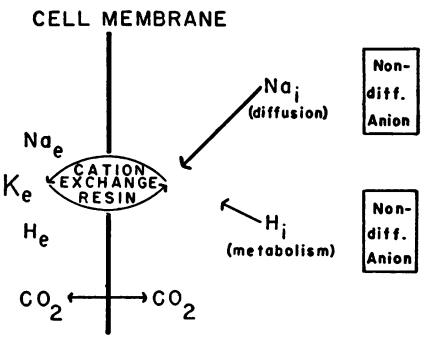

FIG. 2. Proposed mechanism of regulation of cell composition showing competition between intracellular sodium and hydrogen for exchange with extracellular potassium.

centration as calculated from the difference between extracellular sodium and chloride concentrations.[14] In these experiments muscle potassium is elevated and sodium depressed (53mM/100g.FFS and —2.3mM/100g. FFS, respectively).

The high potassium, low sodium content of cells has been attributed to a specific cation exchange system which permits transfer of sodium outward and potassium into the cell.[19] The data presented above suggest that this reaction may be concerned with transport out of the cell of hydrogen ion as well as sodium. This transport is assumed to be an equilibrium reaction to which the Law of Mass Action is applicable. Therefore, a change in concentration of the reactants on one side of the reaction leads to an alteration in the equilibrium point with a change in concentration of the reactants on the other side.

With these considerations it is possible to formulate a theory of electrolyte transfer at the cell membrane which could explain the alterations in muscle composition observed with changes in extracellular electrolyte com-

position. It may be postulated that intracellular sodium (entering the cell by diffusion) and intracellular hydrogen (resulting from cell metabolism) compete with each other at the cell membrane for a cation exchange system. This system, which perhaps has properties like an exchange resin, transfers intracellular sodium or hydrogen outward and extracellular potassium into the cell (Fig. 2). The quantity of sodium or hydrogen which is transported outward in exchange for potassium transported inward depends upon the concentrations of these materials on either side of the reaction. The shift in equilibrium point in this reaction is thought to account for changes in cell composition. It is noteworthy that, in terms of this theory, the loss of potassium from the cell or the movement of sodium into the cell is of little importance as regards cell composition. Since both of these processes take place by passive diffusion, they are dependent simply upon the difference in concentration between cell and extracellular fluid. Passive diffusion, therefore is essentially constant despite large changes in active exchange since the differences in concentration between cell and extracellular fluid are altered little by large percentage changes in extracellular potassium or intracellular sodium concentration. For example, a *50 per cent* fall in extracellular potassium concentration increases the cell to extracellular fluid gradient from 155mM/L (160 (cell)—5 (Excell.)) to 157.7mM/L (160 (cell)—2.5 (Excell.)), a *1.6 per cent* increase. Likewise, intracellular hydrogen ion concentration is considerably higher than extracellular hydrogen ion concentration (pH 6.1 and 7.4, respectively), and is probably maintained at a fairly uniform level because of the constancy of cell metabolism. Thus, the equilibrium point of the reaction, $(K)_e$ for (H)i is more likely to be influenced by changes in extracellular hydrogen ion concentration but may be influenced by alterations of intracellular hydrogen ion concentration resulting from changes in production of (H)i such as those which occur in febrile illnesses and during exercise. It is probable also that hydrogen ion leaves the cell not only by exchanging with potassium but also as H_2CO_3 or H— organic anion. The form in which potassium leaves the cell is considered to be free cation diffusing out as sodium diffuses in. In addition, potassium that enters the cell in exchange for intracellular hydrogen probably leaves the cell with diffusible anion which has resulted from cell metabolism. Otherwise, potassium would accumulate without limit within the cell. Since diffusible anion (probably lactate) is produced at the same rate as hydrogen ion by cell metabolism, the rate of diffusion out of the cell of this anion with accompanying potassium ion is proportional to (H)i for $(K)_e$ exchange. Therefore, at times when $(K)_e$ for (H)i exchange is accelerated, more organic anion would appear in the extracellular fluid.

The maximum concentration of cation which can be attained within the cell is limited by the quantity of intracellular non-diffusible anion. Conway[4] has calculated the concentration of the sum of the non-diffusible anions and has represented this sum in terms of the idiomolar value of cell fluid. It is

these large immobile protein phosphate complexes that are important in maintaining the constancy of the total cation concentration of the body fluids. The relative proportion of various cations in the cell is determined by the transfer mechanism described above.

As can be seen from Figure 2, a change in concentration in any one of the components of this exchange system may modify the equilibrium with alteration in muscle or extracellular composition as a result. Since the extracellular concentrations of both potassium and hydrogen are fractions of their intracellular concentrations, a small absolute change in extracellular concentration of these ions must have a considerably greater effect on the equilibrium than the same absolute change in intracellular concentration. Thus, it is the components of low concentration which are of primary importance in the equilibrium. It must be emphasized that these processes are dynamic equilibria in which changes in composition occur only until a new stable state is achieved with altered concentrations of the reactants on each side of the cell membrane. When this new equilibrium is established, the exchange continues at the same rate as before but body composition remains in the same altered state. This altered composition persists until the addition or removal of a reactant again shifts the equilibrium.

Metabolic Alkalosis

In extracellular metabolic alkalosis the fall in extracellular hydrogen ion concentration increases the gradient of hydrogen ion between cell and extracellular fluid. As a consequence, the exchange of $(H)_i$ for $(K)_e$ is accelerated and more $(H)_i$ is transported into the extracellular fluid. The shift in this equilibrium permits the cell to reduce the magnitude of extracellular alkalosis and accounts for the fact that the serum bicarbonate does not rise as high after the administration of a given amount of bicarbonate as calculated from the extracellular distribution of the sodium of administered sodium bicarbonate.[12] The final intracellular hydrogen ion concentration may not be altered or may actually rise since with the reduced breathing which accompanies extracellular alkalosis, the carbon dioxide concentration rises in all body fluids. If intracellular bicarbonate concentration remains constant, as stated by Wallace and Hastings,[20] intracellular hydrogen ion concentration will rise under these circumstances.

When $(H)_i$ for $(K)_e$ exchange is increased the $(Na)_i$ for $(K)_e$ exchange is reduced, because of the competition between these reactions. If it is assumed that the exchange of intracellular sodium $(Na)_i$ for potassium $(K)_e$ is usually of greater magnitude than the exchange of intracellular hydrogen $(H)_i$ for extracellular potassium $(K)_e$, the net result is that less potassium is transported into the cell, and low intracellular potassium results. High intracellular sodium is, of course, the result of diminished sodium transfer outward. The electrostatic equivalent of the non-diffusible

anions within the cell is thus satisfied by low potassium and high sodium. The diminished transfer of potassium into the cell leads to accumulation of potassium in the extracellular fluid since diffusion out of the cell continues at a fairly constant rate. This accumulation of extracellular potassium might account for the increased potassium excretion in the urine observed in metabolic alkalosis,[13] since the kidney assiduously guards against a rise in extracellular potassium concentration.[15]

Metabolic Acidosis

In extracellular metabolic acidosis the rise in hydrogen ion concentration outside the cell decreases the gradient between cell and extracellular fluid. As a consequence, the exchange of $(H)_i$ for $(K)_e$ is decelerated. Such a shift in this equilibrium permits the cell to reduce the magnitude of extracellular acidosis. The exchange of $(Na)_i$ for $(K)_e$ is accelerated, since the competing reaction, $(H)_i$ for $(K)_e$, is reduced. Since $(Na)_i$ for $(K)_e$ exchange is of greater magnitude than $(H)_i$ for $(K)_e$ exchange, intracellular potassium is increased somewhat and intracellular sodium is depressed.

Respiratory Acidosis

In respiratory acidosis the rise in extracellular hydrogen ion concentration decreases the exchange of $(H)_i$ for $(K)_e$. As a consequence, the exchange of $(Na)_i$ for $(K)_e$ is accelerated and intracellular sodium is slightly low or normal while potassium is slightly high or normal. In respiratory alkalosis the converse occurs.

The small magnitude of the changes in muscle composition in respiratory disturbances is readily explained by the fact that carbon dioxide penetrates cells fairly readily and therefore $(H)_i$ varies directly with $(H)_e$ in these states. The $(H)_i$ for $(K)_e$ exchange is not markedly disturbed and therefore the $(Na)_i$ for $(K)_e$ exchange remains relatively unaffected.

The increased undetermined anion concentrations (lactate) observed in states of low extracellular hydrogen ion concentration (alkalosis) may be explained on the basis of greater potassium entry into the cell in exchange for $(H)_i$ rather than $(Na)_i$ and therefore more potassium leaving the cell, presumably as potassium lactate. The increase in serum lactate and fall in muscle potassium observed in exercise is also consistent with this concept since greater $(H)_i$ production would occur under these circumstances.

Potassium Deficiency

In those states in which potassium is lost from the body and the concentration of extracellular potassium falls, there is diminished exchange of both $(Na)_i$ and $(H)_i$ for $(K)_e$. As a result, intracellular potassium falls and intracellular sodium rises. Because of the diminished transfer of hydro-

gen out of the cell, extracellular bicarbonate rises (Fig. 3).[8] In effect the cell adds to the extracellular fluid one fixed cation in excess of fixed anion for each hydrogen ion which accumulates within the cell as the net result of altered transfer. The kidney evidently does not excrete this load of cation without some increased loss of chloride in the urine. This loss of chloride leads to the hypochloremia that is associated with alkalosis in potassium deficiency. It has not been adequately established that the kidney is able to conserve chloride, after bicarbonate loading, less efficiently in potassium deficiency than under normal circumstances.

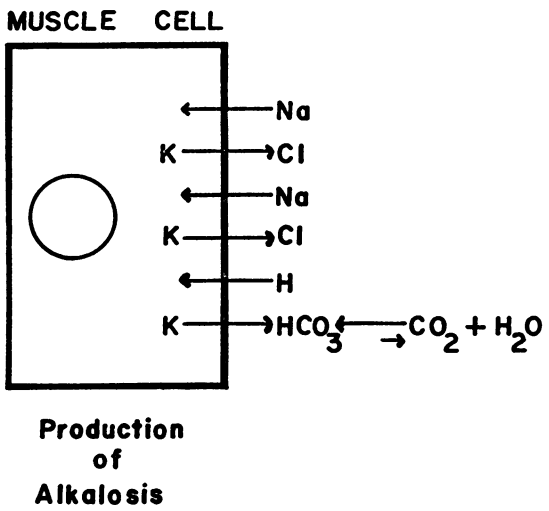

FIG. 3. Mechanism of production of alkalosis in potassium deficiency.

Why the kidney does not compensate for the load of fixed cation in excess of fixed anion which comes from the cells is not known. If the changes in composition of muscle, which are described in potassium deficiency, apply also to the cells of the distal tubule of the kidney, high intracellular hydrogen and low potassium concentration would be present. These findings might account for increased urinary secretion of hydrogen ion in potassium deficiency as postulated by Berliner.[1]

It is of interest to note that if the changes in muscle composition described in this paper apply also to the kidney, the tubular cells of the kidney should show high intracellular hydrogen ion concentration in metabolic and respiratory acidosis and low intracellular hydrogen ion concentration in metabolic and respiratory alkalosis. Urines of low pH would be expected in acidosis and urines of high pH in alkalosis. It is only in the alkalosis associated with potassium deficiency that high intracellular hydrogen ion concentration might be present. Therefore, renal correction of alkalosis in potassium deficiency might be expected to be limited. However, no significant alterations in water and electrolyte content of kidneys of rats, which would confirm these postulations, were noted in potassium deficiency.[9] Small changes in specific cells could not be determined by the method of organ analysis that was used.

The fact that the change in intracellular sodium in potassium deficiency is about twice the change in intracellular hydrogen as derived from determination of muscle sodium and potassium ($\Delta (Na)_i = -\frac{2}{3} \Delta (K)_i$, the

other one-third being Δ $(H)_i$) indicates that the $(Na)_i$ for $(K)_e$ exchange is usually of greater magnitude than the $(H)_i$ for $(K)_e$ exchange. Since $(K)_e$ exchanges for both $(Na)_i$ and $(H)_i$ the reduction of $(K)_e$ would be expected to reduce the transfer of $(Na)_i$ and $(H)_i$ proportionately. For example, if it is assumed that $(Na)_i$ for $(K)_e$ exchange takes place at the rate of 100mM per minute and $(H)_i$ for $(K)_e$ exchange occurs at 50mM per minute and that both reactions are reduced 10 per cent by a fall in $(K)_e$, then, in absolute quantities, the retention of $(Na)_i$ would be twice the retention of $(H)_i$.

In extracellular alkalosis accompanying potassium deficiency, severe intracellular acidosis should be present, since the original disturbance was due to deceleration of hydrogen ion transfer out of the cell. In addition, respiratory compensation of the extracellular alkalosis leads to elevated pCO_2 of the body fluid which further increases intracellular acidosis.

Potassium Excess

The converse of the above processes probably holds true in potassium excess and accounts for the fall in extracellular bicarbonate concentration, the rise in intracellular potassium, and the fall in intracellular sodium which are noted in this condition.[14]

Summary

In summary, the transfer of electrolyte between cell and extracellular fluid and alterations in this transfer may be accounted for in simple physical chemical terms involving the application of the Law of Mass Action. Instead of complex mechanisms requiring specific regulation, the constancy of the cation concentration of muscle would seem to depend in large part upon the constancy of three factors:

1. The intracellular non-diffusible anion concentration is maintained at a stable level through cell anabolism. The absence of variability in muscle phosphorus in many different states (Table 1) is an example of the constancy of these anions. This quantity of anion limits the maximum concentration of cation which can be maintained within the cell. Changes in valence of the phosphate protein complexes can occur only with change in intracellular hydrogen ion concentration. Therefore, with alterations in hydrogen ion exchange, valences of these compounds may be altered (and total intracellular fixed cation concentration may be affected). Because of the free diffusion of water, extracellular concentration of cation parallels that of intracellular cation.

2. The extracellular potassium concentration is regulated within narrow limits by renal transport mechanisms. Thus, alterations in renal function may markedly influence intracellular composition, since the exchange of $(Na)_i$ and $(H)_i$ for $(K)_e$ is altered by change in extracellular potassium concentration.

3. The extracellular hydrogen ion concentration is maintained with little variation by respiratory and renal regulatory mechanisms operating through the buffer systems of the body. The proper and great significance of the extracellular buffers can now be understood since they resist change in extracellular hydrogen ion concentration. This constancy of the extracellular hydrogen ion concentration exerts profound influence on the total body composition by preventing alteration in the exchange of electrolyte between cell and extracellular fluid.

By means of the hypothesis presented in this paper it is possible to predict body electrolyte composition in a variety of disturbances and the data so far obtained from tissue analysis agree with these predictions. This hypothesis in no way conflicts with existing knowledge of the rôle of kidney, lung, or buffers in the regulation of the internal environment of the body but includes them in a dynamic framework operating according to simple thermodynamic principles.

The significance of these concepts is obvious. Body function is dependent upon the transfer of energy within cells and the chemical reactions which release energy depend solely upon the concentrations of reactants, coenzymes, inhibitors, etc. within the cell. The composition of the fluid outside of the cell is of importance only in its effect on composition within the cell. Therefore, since parenteral fluids must be introduced into the extracellular space alone, the fluids administered should alter extracellular concentrations in a way that will correct deviations in intracellular composition.

ACKNOWLEDGMENT

The authors are indebted to Dr. Daniel C. Darrow for his advice and guidance in the preparation of this paper.

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