Kidney International, Vol. 11 (1977), pp. 433–442

Potassium and intracellular pH

SHELDON ADLER and DONALD S. FRALEY

Department of Medicine, University of Pittsburgh School of Medicine and Montefiore Hospital, Pittsburgh, Pennsylvania

Potassium and acid-base balance have long been known to be interrelated. Studies have shown, for example, that a reduction in body potassium stores often induces a sustained metabolic alkalosis [1, 2], while acute administration of either potassium or rubidium chloride results in a rapid fall in blood pH and blood bicarbonate concentration [3]. The vast majority of metabolic reactions, however, occur intracellularly, so the effect of changes in potassium balance on cellular acidity potentially have even greater physiologic significance. Most experiments dealing with this topic suggest that potassium balance does indeed influence cellular acid-base conditions. The present paper will review the information available concerning the relationship between potassium and cell pH and indicate those areas in which the data are incomplete or the results ambigious. As much work in this field is dependent on the accurate measurement of cell pH, it is necessary to examine briefly the practical and theoretical limitations of the methods employed to determine this quantity.

Measurements of cell pH

All methods for the measurement of cell pH suffer certain basic theoretical problems which make interpretation of results difficult. A major difficulty is the definition of the entity entitled "cell pH." It is apparent that the cell is nonhomogeneous with respect to pH, being composed of many areas of varying pH. Thus, cytoplasmic pH differs from the pH within organelles such as mitochondria [4]. Also, there are pH differences which occur across the surface of protein molecules [5]. The value called cell pH, therefore, is either some mathematical mean relating each of these pH areas or is a measurement of a single portion of the cell. The term pH heterogeneity has been coined to denote this problem [6]. It follows that cell pH measurements may vary according to the method used since different quantities will be measured. Furthermore, the relationship between cell pH and metabolism is often difficult to ascertain, as the change in overall or mean cell pH may not reflect a change of pH in the area of the cell wherein the reaction takes place.

Another major problem with cell pH measurements relates to the effect of the method of measurement upon the cell pH itself. Electrodes, for example, may induce cellular injury and alter the pH, while weak acids or bases used for the calculation of cell pH may influence cellular metabolism and intracellular acidity. Other measures suffer from similar limitiations. These difficulties are enhanced by the ability of cells rapidly to produce or consume protons in response to changes induced by the measurement. Not only are these changes rapid, but they may occur in one particular portion of the cell, thereby generating local areas of relative acidity or alkalinity. With these general considerations in mind, the seven methods currently employed to measure cell pH will be examined individually and the theoretical limitations of the most widely used methods detailed.

Cytoplasmic sampling. This method involves direct removal of cytoplasmic fluid by micropipette and measurement of the fluid by standard methods. It is useful only for large cells such as the giant barnacle [7] and has not been employed to any significant extent in mammalian tissue.

Colored indicators. Indicators such as bromthymol blue have been used to measure the pH of the muscle of the giant barnacle and roughly to estimate changes in mitochondrial pH [8]. Injection of this dye into frogs *in vivo,* excision of the semitendinosus muscle followed by incubation and examination of the muscle *in vitro* has also been performed [9]. This method, however, can only detect gross changes and has not been used to study mammalian tissues to any significant extent.

Cell lysis. This is a limited method, useful only in non-nucleated red blood cells. Lysis is accomplished by successive freezing and thawing, followed by measurement of the pH of the lysate by a pH-sensitive glass electrode. As this method has not been used to measure cell pH in abnormal potassium states, further discussion is not necessary.

Nuclear magnetic resonance. Although this method may be employed to advantage in the future, at the present time it has only been used to measure the pH of red blood cells [10]. A host of technical problems preclude its use in other mammalian tissues, so no work on cell pH and potassium metabolism has been performed utilizing this technique.

Mass action method. In this method intracellular pH is calculated from the measured quantities of an intracellular reaction in which one of the reactants is the hydrogen ion. There are theoretical as well as practical limitions to this method. The mass action constant under cellular conditions must be assumed, and the reactants must truly be intracellular. This method appears to hold some promise in the future, but as has been the case with the preceding four methods, it has not been extensively used in mammalian tissues [11].

Microelectrode method. This is one of the two most widely used methods for measuring the intracellular pH of mammalian tissues. Indeed, it frequently serves as a reference to check the validity of other methods. The type of electrodes used include pH-sensitive glass electrodes [12], antimony electrodes [13], and bicarbonate-selective electrodes [14]. Technical problems arise due to the necessity of having adequate insulation of the electrode above the pH-sensitive tip. Should any part of the uninsulated measuring portion lie outside the cell, the pH measured will reflect changes both in extracellular pH and in the membrane potential [15]. Insertion of the electrode may also damage the tissue, giving rise to an injury current or to changes in metabolic reactions which produce or consume proteins. An example of the latter is the synthesis of adenosine triphosphate from adenosine diphosphate. Most important, the electrode measures the pH within only a single portion of the cell. As there is significant cell pH heterogeneity, the quantity being measured by the electrode is often difficult to evaluate. This problem has been examined critically in two recent reviews [7, 11].

Weak acid or weak base distribution method. This is the most extensively used method for measuring cell pH in mammalian tissue and is the one which has been employed in the majority of studies of cell pH in abnormal potassium states. The theory involved in this method has been well described [11, 16]. Briefly, it depends upon the observed fact that a weak acid will accumulate within areas of alkalinity, while a weak base will accumulate in those areas which are acidic. This depends upon the greater permeability of the unionized species of the conjugate acid-base pair and its greater ability to move across membranes relative to the movement of the ionized portion of the conjugate pair. The two weak acids commonly employed are carbonic acid (total carbon dioxide or carbon dioxide/bicarbonate system) and 5.5-dimethyl-2,4-oxazolidinedione (DMO). Weak bases used have included ammonia and nicotine. Problems occurring with this method include high permeability of the cell membrane to the ionized species (poor selectivity), the assumption that the dissociation constant of the acid or base is the same in the intracellular and extracellular compartments, protein binding of the marker, metabolic conversion of the acid or base, active transport of these compounds, and difficulties in accurately measuring the extracellular space. Even when these problems are overcome, the value determined by the method is not a true mean pH. Rather, it represents an algebraic sum of the different areas of pH within the cell [16, 17]. Indeed, measurement of muscle cell pH simultaneously using either a weak acid method (DMO) or a weak base method (nicotine) revealed marked differences in the calculated overall cell pH value [6]. Nevertheless, this method does appear to be reliable for the study of cell pH in mammalian tissue so long as the problems inherent in it are considered and dealt with. The method is especially useful in showing the direction of overall change of cell pH when perturbations are applied to a single system. Thus, the cell pH of a tissue may be measured in the normal state and this value compared to one obtained in an abnormal state, such as hypo- or hyperkalemia. Unfortunately, the method is difficult to apply to a heterogeneous tissue such as kidney which actively transports and accumulates weak acids and bases. The majority of studies have, therefore, dealt primarily with skeletal muscle, or less frequently studies have been performed in cardiac muscle, liver, or renal tubules.

Relationship between intracellular pH and potassium distribution within the body

It has long been appreciated that the concentration of cellular potassium is somehow dependent upon and related to the level of intracellular acidity. This belief stems from the experiments of Fenn and Cobb [18], who reported in 1934 that when the pH of a saline bathing medium was lowered by raising the carbon dioxide tension of the system, potassium

moved from the skeletal muscle into the medium. When the experiment was repeated using blood instead of saline as the bathing medium, however, potassium was found to move in the opposite direction, i.e., from the medium into the tissue. The authors postulated that as muscle tissue is better buffered than saline but less buffered than blood, lowering the pH of the saline solution resulted in a smaller fall in intracellular pH relative to the bathing medium, while the converse occurred when blood was used to incubate the tissue. This gave rise to the concept that some mechanism exists, possibly a Donnan equilibrium, maintaining rough equality between intracellular and extracellular hydrogen ion and potassium ion ratios [17]. According to this hypothesis, if the hydrogen ion activity of the extracellular fluid (H^+_{EXT}) increased to a greater extent than intracellular hydrogen ion activity (H^+_{INT}), the fall in the H^+_{INT}/H^+_{EXT} ratio would be reflected by a rise in extracellular potassium (K^+_{EXT}) and a decrease in the K^+_{INT}/K^+_{EXT} ratio. Data supporting this suggestion were subsequently obtained in both dog and man, showing that a decrease in extracellular pH raised the plasma potassium concentration [19, 20]. In one large study in man, extracellular potassium was higher in metabolic acidosis than in respiratory acidosis [21], a result predicted by this hypothesis since most experiments have shown a greater decrease of cell pH in respiratory than in metabolic acidosis [17]. This should result in a greater decrease in the H^+_{INT}/H^+_{EXT} ratio in metabolic acidosis than in equal degrees of respiratory acidosis. In none of the foregoing experiments, however, was cell pH determined. Brown and Goott [22], using the DMO technique, directly calculated the H^+_{INT}/H^+_{EXT} ratio in dog skeletal muscle under a variety of external acid-base conditions. They showed that in almost all instances a decrease in this ratio (acidosis) was accompanied by a rise in plasma potassium concentration, while an increase in the ratio (alkalosis) resulted in a decrease in plasma potassium concentration. These changes were only directionally but not quantitatively valid, and experiments with Tris buffer gave conflicting results. In 1969 Waddell and Bates [17] reviewed the data then available in rat, cat, dog, and man concerning the relationship of cell pH to potassium distribution across the skeletal muscle membrane. All studies utilized either the carbon dioxide or DMO weak acid distribution methods. They demonstrated a linear relationship between the logarithmic change in the intracellular to extracellular potassium concentration and the intracellular to extracellular pH gradient. A similar relationship was demonstrable for sodium distribution, suggesting that common factors are responsible for determining changes in the ratios of these three ions. Since the change in the concentration gradient of sodium was five times larger than the alterations in potassium and hydrogen ions, the authors felt this suggested intracellular binding of the sodium ions. No specific data relating to this point, however, were presented. It is also important to remember that measurement of intracellular sodium is prone to a great deal of error because of its primary extracellular location.

Although a relationship between the H^+_{INT}/H^+_{EXT} and K^+_{INT}/K^+_{EXT} ratios seems definitely to exist, it is by no means a constant one, and the exact relationship between cell pH and transcellular potassium distribution appears to be extremely complex. This conclusion follows from a number of factors. First, when the data described in the preceding section are closely examined, they exhibit a great deal of scatter. In rats, for example, a 0.15 change in the logarithmic K^+_{INT} ratio occurs over a pH gradient change ranging all the way from 0.10 to 0.50 [17]. Similarly, there is much scatter in the data obtained from dogs. Insufficient data are available from either cat or man to ascertain their degree of reproducibility. Second, as has already been discussed, the measurement of cell pH at the present time has many theoretical limitations, and the effect of cell pH heterogeneity is impossible to ascertain. Third, there are dramatic differences between individual tissues. Thus, in prolonged respiratory acidosis, potassium moves into cardiac tissue [23] at the same time that it moves out of skeletal muscle [24]. Yet, most data indicate that the pH of cardiac muscle decreases in respiratory acidosis in a fashion similar to skeletal muscle [25] so that the cardiac H^+_{INT}/H^+_{EXT} ratio falls. This should lead to an efflux of potassium from cardiac tissue if cellular potassium concentration was solely determined by ionic ratios and/or passive Donnan forces. The converse has also been shown, so that restoration of normal acid-base conditions after prolonged respiratory acidosis causes potassium to move in opposite directions in skeletal and cardiac muscle [24, 26]. Fourth, recent data are now available demonstrating that potassium distribution across the skeletal muscle membrane can be dissociated from the H^+_{INT}/H^+_{EXT} ratio. Kim and Brown [27], using the DMO technique, studied this problem in nephrectomized dogs and showed that volume expansion in these animals, induced by saline or mannitol infusion at a constant extracellular pH, raised the external potassium concentration despite constancy of the pH gradient.

The earlier work of some investigators [28, 29] suggested that alterations in potassium movement produced by changes in acid-base balance of respi-

ratory origin correlate more closely with the pH change than those produced by metabolic alterations. This would now appear to be at least partially explained by several alterations which are independent of the changes in acidity, produced experimentally by the infusion of acid or alkaline solutions. These "metabolic" alterations include an increase in extracellular fluid volume, a dilution-produced decrease in the concentration of extracellular electrolytes, or even hemodilution itself [27]. Other investigators [30, 31] have shown that extracellular potassium concentration is increased after the infusion of saline solutions. This had been attributed to the acidosis resulting from the dilution of extracellular bicarbonate occurring without a corresponding fall in carbon dioxide tension. Examining this point specifically Makoff, da Silva, and Rosenbaum [32] demonstrated that during volume expansion by hypertonic infusion of sodium chloride or mannitol, extracellular pH falls while intracellular pH rises. Thus, whole body intracellular pH (in essence, skeletal muscle pH) increased by 0.25 pH units at a time when plasma osmolarity had risen between 40 and 80 mosmoles/kg of water. By adding variable amounts of sodium bicarbonate to regulate changes in extracellular pH, these investigators were able to demonstrate that the resulting degree of hyperkalemia correlated inversely with the fall in extracellular pH produced by the infusion. As intracellular pH always rose, the $H^{+}{}_{INT}/H^{+}{}_{EXT}$ ratio fell in each of the experiments. Yet, in ten of the fourteen studies, the $K^{+}{}_{INT}/K^{+}{}_{EXT}$ ratio increased, clearly showing that changes in these two ionic ratios may be dissociated. In contrast, Irvine and Dow had [33] observed a negative exponential relationship between the intracellular pH of skeletal muscle and plasma potassium in nephrectomized rats made acidotic with ammonium chloride and a direct linear relationship between hydrogen and potassium ion gradients. Adler [34], however, utilizing an *in vitro* intact rat diaphragm muscle preparation and measuring cell pH from both weak acid (DMO) and weak base (nicotine) distributions, was able to dissociate the hydrogen ion and potassium ion ratios. Potassium ion ratios were altered by acutely depleting the diaphragms of potassium *in vitro* by incubation in a zero potassium medium. Although the $K^{+}{}_{INT}/K^{+}{}_{EXT}$ ratio increased tenfold from 30 to 300, muscle cell pH was unaffected if external pH was maintained constant at 7.40.

The simplest explanation at this time for the commonly observed association of hyperkalemia and extracellular acidosis appears to be that the ratios of $K^{+}{}_{INT}/K^{+}{}_{EXT}$ and $H^{+}{}_{INT}/H^{+}{}_{EXT}$ do indeed change concomitantly in this condition. The rise in extracellular hydrogen ion activity is accompanied by a smaller but proportional rise in intracellular hydrogen ion activity and a fall in the hydrogen ion ratio. Decreased cell pH would then lead to a decrease in the ionization of weak anionic groups within the cell followed by the extrusion of potassium secondary to the charge imbalance. Active transport of hydrogen ions, bicarbonate ions, or potassium ions, however, could upset this simple balance. Thus, entry of bicarbonate into the cell could reduce the extrusion of cell potassium. Recent experiments [35] showing that under external isohydric conditions potassium moves from the cells into the extracellular compartment in either hypokalemic, normokalemic, or hyperkalemic rats when external bicarbonate concentration is lowered support this possibility. Interestingly, these investigators were able to show that when bicarbonate concentration was raised, potassium did indeed enter the cells. It seems fair to conclude at this time that intracellular pH is one of the important determinants of cellular potassium concentration, but the precise relationship between these two entities is not a simple linear or logarithmic one. Better methods for the measurement of cell pH and free intracellular potassium and sodium are clearly necessary.

Effect of potassium depletion upon intracellular pH

The extracellular metabolic alkalosis which accompanies potassium depletion has been widely investigated over the past two decades. It has variously been attributed to chloride depletion [36, 37], altered intracellular pH [38], contraction of the extracellular space [39], or some combination of these various factors. It appears now that chloride depletion is principally responsible for the vast majority of clinically occurring metabolic alkaloses not associated with steroid excess [40, 41]. In addition to the changes in extracellular acid-base balance, it is apparent that cellular pH is also altered in this state. Skeletal muscle, being both the largest readily available body buffer pool and the tissue in which pH may most easily be measured, has been most extensively studied. Early experiments by Cooke et al [1] demonstrated that in potassium deficient rats the gain of potassium by the skeletal muscle during potassium repletion was greater than the loss of sodium. They postulated that hydrogen ions must have been exchanged to preserve electroneutrality and that in potassium depletion hydrogen ions move from the extracellular compartment into the muscle, while during potassium repletion these hydrogen ions leave the cell and return to the extracellular compartment. They postulated that cell pH must, therefore, be reduced in the potassium-depleted state. This con-

clusion does not, however, follow from their experimental data. Basic amino acids such as lysine, for example, have been shown to accumulate in rat skeletal muscle during potassium depletion [42] and could account for the measured cationic imbalance. Furthermore, alterations in the cellular binding of sodium and potassium, alterations in the amount and type of intracellular phosphates, or changes in the cellular production or consumption of organic acids accompanying the potassium-depleted state could explain the results of Cooke et al in the absence of a change in cell pH. Indeed, direct glass electrode measurement of the pH of muscle homogenates obtained from potassium-deficient rats showed only a small reduction in pH [43]. Subsequent work, however, has confirmed the original hypothesis of Cooke et al that muscle cell pH is decreased in potassium depletion. Direct measurement of skeletal muscle pH *in vivo* in potassium-depleted rats, using the tissue carbon dioxide method [44], the DMO method [38], or by direct measurement of cellular bicarbonate concentration using an ion selective microelectrode [14], have each shown intracellular acidosis in this condition. The change in skeletal muscle cell pH in potassium depletion is not restricted to rats, but the results in other species are less convincing. Wilson and Simmons [45], using the DMO method, measured skeletal muscle pH in potassium chloride-depleted dogs and found it to be reduced. In contrast to other investigators who had found a fall in the sum of intracellular sodium and potassium in potassium-depleted rats [46], Wilson and Simmons showed an increase in this sum in their potassium-depleted dogs. Since extracellular sodium concentration fell in all their experiments, they suggested that the apparent osmotic and electrical imbalance caused by the opposite movements of the extracellular and intracellular cations was best accounted for by conformational changes in intracellular proteins and the appearance of new anionic sites or, alternatively, by the binding of intracellular sodium or potassium. It is important to recognize, however, that despite the apparent fact that in potassium depletion the sum of intracellular sodium and potassium increased in this experiment in the dog while it decreased in the rat, in both species skeletal muscle cell pH seems to be decreased. Grantham and Schloerb [47], using the ^{14}C-DMO method, also found a decrease in the muscle cell pH of potassium-depleted dogs. Conflicting data in the dog, however, have been reported by Burnell and Dawborn [48]. Using the DMO technique, they were unable to measure any fall in muscle cell pH in either potassium chloride or purely potassium-depleted dogs. Consonant with other data [47], they did show a decrease in the buffering capacity of the potassium-depleted muscle. At present there is no explanation for these disparate results in the dogs. In addition to rat and dog, it has also been shown that rabbit skeletal muscle pH is decreased in potassium depletion [49]. In almost all of the experiments cited, potassium depletion was accompanied by chloride depletion, so the effect of pure potassium deficiency was not determined. Wilson and Simmons [45], however, restored the chloride deficit and observed that although extracellular alkalosis was eliminated the intracellular acidosis remained. They felt that intracellular acidosis was specifically related to potassium deficiency rather than the accompanying chloride depletion. This will be discussed in more detail subsequently in this article.

In contrast to the *in vivo* studies in rats, initial *in vitro* work did not confirm that muscle cell pH was altered in potassium depletion. In addition to the muscle homogenate measurements already alluded to, Miller, Tyson, and Relman [50], using both the tissue carbon dioxide and DMO methods, measured the pH of isolated resting diaphragm muscle depleted of potassium *in vitro* and were unable to show any reduction in pH. The apparent conflict between the *in vivo* and *in vitro* data seems to have been resolved by some recent work of Adler, Zett, and Anderson [38]. These investigators, also employing the isolated resting diaphragm muscle model, measured cell pH during *in vitro* potassium depletion using the distribution of either ^{14}C-DMO or ^{14}C-nicotine and compared their results to data obtained in non-potassium-depleted diaphragms. Figure 1 depicts their results when extracellular pH was varied by altering the carbon dioxide tension of the medium. It can be seen that at an external pH value of approximately 7.40 the intracellular pH of potassium-depleted tissue was only slightly lower than that of non-depleted muscle. However, at alkaline and especially acid external pH values, muscle cell pH in the potassium-depleted diaphragms was significantly lower than in the non-potassium-depleted tissue. Similar results were shown by these investigators when medium pH was altered by changing the external bicarbonate concentration. The authors concluded that these data helped toward resolving the apparent contradiction between the *in vitro* and *in vivo* results. Others have pointed out that potassium depletion *in vivo* is usually associated with an elevated carbon dioxide tension [51, 52], a situation in which muscle cell pH is lower during potassium depletion [53]. Indeed, in the *in vivo* experiments in which the carbon dioxide titration curve in potassium depletion was compared to normal, buffer capacity was found to be reduced in the former state

[51, 52]. When carbon dioxide tension and external pH approach 40 mm Hg and 7.40, respectively, *in vitro,* then cell pH in potassium-depleted and non-potassium-depleted tissue becomes similar—a result consistent with only small changes in cell pH potassium depletion at "normal" carbon dioxide tensions. Since Miller, Tyson, and Relman [50] performed their experiments at a single external pH value of 7.40 mm Hg, this probably explains why they were unable to measure any change in muscle cell pH. Regardless of the exact cell pH value, it seems certain that skeletal muscle buffering capacity is reduced both *in vitro* and *in vivo* in the potassium-depleted state and that addition of acid to such tissue or to the animal could result in a severe reduction in intracellular pH with possibly severe metabolic consequences.

The effect of potassium depletion upon the pH of other tissues has been less thoroughly investigated. Cardiac muscle, especially the left ventricle, is much better buffered than is skeletal muscle [49]. Larson and Burnell showed that potassium depletion in dogs led to a reduction in the potential buffering capacity of both skeletal and cardiac muscle. Hall and Cameron measured the cell pH of heart and skeletal muscle in potassium-depleted rabbits [49]. Although they found a sharp decrease in skeletal muscle potassium and pH, they could demonstrate neither changes in potassium nor pH of the left ventricular muscle. Atrial and right ventricular muscle potassium decreased, but intracellular pH did not change. They felt this was due to the greater buffering capacity of cardiac muscle as compared to skeletal muscle. Renal tubular cell pH in potassium depletion has been measured *in vitro* by a single group of investigators [54]. They were unable to demonstrate any change in this value either during *in vitro* potassium depletion or after potassium repletion. The renal metabolic changes which occur in the potassium-depleted state and which have been attributed by some authors to changes in cell pH will be discussed in a later section of this paper. It should be noted that changes in renal potassium content and liver potassium content in potassium depletion are much less marked than in skeletal muscle [55]. Whether this would predict differences in the acid-base behavior of these tissues in the potassium-depleted state is at this time impossible to state.

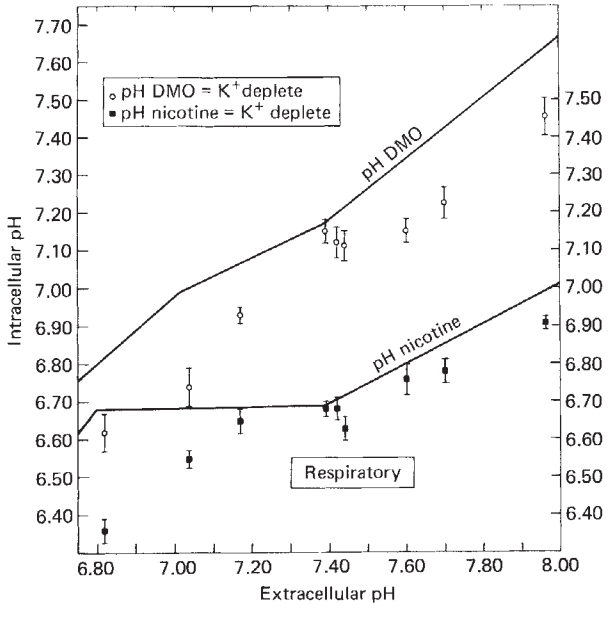

Fig. 1. *Effect of potassium depletion on cell pH compared to that of nonpotassium-depleted tissue as a function of varying extracellular pH by alterations in bath carbon dioxide tension at a constant bicarbonate concentration.* The *solid lines* indicate the relationship previously reported for nonpotassium-depleted tissue at the same fixed bicarbonate concentration. *Points* are the values obtained in the present study with potassium-depleted tissue, and each is the mean ± SD of six analyses (from Adler, Zett, and Anderson [38] with permission of the authors).

Separate roles of chloride and potassium upon intracellular pH

Atkins and Schwartz [40] and Kassirer and Schwartz [41] demonstrated that the administration of chloride in many potassium chloride deficient metabolic alkaloses either partially or completely returns extracellular pH and bicarbonate concentrations to normal, despite the presence of continued potassium deficiency. These studies emphasize the primary role of chloride for restoration of normal extracellular acid-base conditions in hypokalemic states. It seems clear that the majority of clinically occurring metabolic alkaloses are chloride-responsive. Indeed, Burnell, Teubner, and Simpson [56] have demonstrated mild metabolic acidosis in dogs made potassium but not chloride deficient. It has been known for many years from the work of other investigators [57-59], however, that there are cases of saline-resistant hypokalemic metabolic alkalosis, particularly in hyperadrenal conditions. Recently, Garella, Chazan, and Cohen [60] described a group of severely potassium-depleted patients whose metabolic alkaloses were not correctable by saline and who continued to lose chloride in the urine. The alkalosis persisted until large amounts of potassium had been replaced. Thus, there are both patients and animals in whom normal extracellular acid-base conditions can only be restored by administration of both potassium and chloride ions, indicating that both are involved in the maintenance of the extra-

cellular metabolic alkalosis found in potassium deficiency. It has been theorized, therefore, that each ion affects acid-base balance differently. Chloride presumably exerts its major effect through increasing extracellular volume [39], thereby decreasing renal hydrogen ion excretion. Although little data are available on the effect of pure chloride deficiency on cell pH, Khuri et al [14] measured muscle pH in chronically chloride-depleted rats. The rats exhibited a mild metabolic alkalosis, but muscle cell pH and bicarbonate concentration were normal, implying that chloride ions *per se* have no direct effect on cell pH.

Potassium depletion purportedly reduces tissue buffer capacity and increases intracellular acidity. The evidence for this latter hypothesis is suggestive but not definitive. In severely potassium and chloride deficient rats, correction solely of the chloride deficit may only partially correct the extracellular alkalosis [59, 61] induced by DOCA and low potassium ingestion. Rats with pure potassium deficiency, induced by feeding a low potassium, normal chloride diet, exhibit an extracellular alkalosis and a simultaneous decrease in muscle cell pH as measured from the distribution of DMO [55]. In dogs, one investigator induced both potassium and chloride deficiency and found an extracellular alkalosis and a reduction in skeletal muscle pH [45]. When chloride was given to these dogs, the extracellular alkalosis was corrected, but the intracellular acidosis was unaffected. Adler, Zett, and Anderson [55] showed that repletion of chloride ions by administration of sodium chloride to potassium- and chloride-depleted rats partially reduced elevated extracellular bicarbonate levels, but urinary ammonia excretion increased and urinary citrate excretion decreased, a result normally seen in acidosis. The authors postulated a further decrease in an already reduced renal tubular cell pH, but measurements of muscle or renal tubular cell pH were not made. *In vitro,* acute potassium depletion of rat mucle with adequate chloride in the bathing medium also reveals an intracellular acidosis and reduced tissue buffering capacity [38]. In contrast to these findings, Burnell and Dawborn [48] were able to restore both extracellular and intracellular acid-base conditions to normal with chloride given to potassium and chloride deficient dogs. Also, Sansalone and Muntwyler [46] were unable to relate the degree of intracellular acidosis to the degree of potassium depletion within skeletal muscle. The rats they studied, however, were protein deficient, and it is difficult to extrapolate from their data to the nonprotein deficient state. Thus, most results reported do indicate that potassium deficiency, even in the absence of a chloride deficit, does cause an intracellular acidosis in rats, but the extent of the acidosis is difficult to ascertain. The data in dogs are less convincing, but decreased potassium does seem to reduce tissue buffering capacity.

The relationship between hyperkalemia and intracellular pH

Since hypokalemia seems to lower muscle cell pH both *in vivo* and *in vitro,* investigations have been performed to determine whether raising extracellular potassium concentration also affects skeletal muscle acidity. Early work utilizing acute hyperkalemic models demonstrated that when potassium or rubidium chloride was given acutely to normal or potassium deficient animals, extracellular bicarbonate was reduced producing a metabolic acidosis [1, 62]. Subsequent experiments [63] showed that the acute administration of either potassium or rubidium chloride to normal rats caused metabolic acidosis and a simultaneous transient alkalosis in skeletal muscle, as determined by the tissue carbon dioxide method. The authors postulated that the administered cations were exchanged for intracellular hydrogen ions, thereby explaining the simultaneous appearance of an extracellular acidosis and an intracellular alkalosis. Muscle cell pH returned to normal by six hours after the acute administration of either cation. Burnell and Dawborn [48] were unable to demonstrate a reduction in skeletal muscle pH in their potassium- and chloride-depleted dogs. Nevertheless, they found that skeletal muscle pH rose from 6.87 to 7.00 when these depleted dogs were given potassium chloride acutely. Potassium chloride-depleted rats, given either potassium chloride or rubidium chloride, also acutely show a rise in muscle cell pH [63]. Dua et al [64] administered potassium chloride i.v. to normal dogs. These animals developed an extracellular acidosis, an intracellular alkalosis, and a paradoxical bicarbonate diuresis. Although tissue potassium was not measured in this study, the percent increase in tissue potassium concentration must certainly have been less than the increase in extracellular potassium concentration. The intracellular to extracellular ratio of these two ions would, therefore, have fallen as did the intracellular to extracellular hydrogen ion ratio. Whether these two changes are causally related cannot be determined from the data presented. *In vitro* data support the results found *in vivo.* Intact rat diaphragms, either potassium-depleted or non-potassium-depleted, when incubated in a bathing medium containing an elevated potassium concentration, show an increase in cell pH, as measured by either the DMO or tissue carbon dioxide methods [50]. No data are available showing the effect of

chronic hyperkalemia on cell pH. Chronic potassium-loading in rats was performed by Khuri, Agulian, and Bogharian [14]. Unfortunately, no values for serum potassium concentration were given. In addition, the rats exhibited not a metabolic but a respiratory acidosis. Measurement of muscle cell pH by an ion-specific bicarbonate microelectrode in these chronic potassium-loaded rats did reveal an increase in intracellular bicarbonate concentration, but cell pH was normal, presumably due to the elevated carbon dioxide tension. Since elevated carbon dioxide tension alone [65] raises the cellular bicarbonate concentration, it is impossible to relate the alteration in cell bicarbonate to the chronic potassium-loading.

The data available on the effect of *in vivo* potassium depletion and acute hyperkalemia upon skeletal muscle pH are summarized in Figure 2. It is obvious that there is a complete separation between these two conditions with intracellular pH always being higher in hyperkalemia. Although there is some scatter in the potassium depletion data, it seems fair to conclude at this time that acute hyperkalemia raises muscle cell pH, while chronic potassium depletion lowers it. As has been discussed previously, the exact mechanism through which these changes occur is not well understood.

Interrelationship between abnormal potassium levels, altered cell pH, and metabolism

Given the preceding evidence that changes in potassium concentration affect intracellular acidity, it is necessary to ask what is the metabolic and physiologic importance of this effect. As pH is a potent regulator of metabolism, it has been widely assumed that many of the metabolic changes seen in abnormal potassium states are due to altered cellular acidity. It is well known, for example, that in potassium depletion urinary ammonia excretion is elevated [66] while urinary citrate excretion is decreased [67]. This response is the one usually found in acidosis, but these animals are alkalotic. Altered renal metabolism has, therefore, been ascribed to a reduction in renal tubular cell pH [55] analogous to the intracellular acidity measured in potassium-depleted skeletal muscle. Hudson and Relman [63] demonstrated that the transient intracellular alkalosis found after rubidium or potassium chloride administration was associated with a rise in muscle citrate content. They postulated that the change in muscle citrate metabolism was due to the cation-induced rise in pH. The only direct evidence which relates altered tissue metabolism to a change in cell pH induced by potassium depletion has been obtained *in vitro*. Adler, Anderson, and Zett [68] measured the cell pH and citrate content of intact rat diaphragm muscle acutely potassium-depleted *in vitro*. They showed that changes in muscle citrate content were best correlated with cell pH and not muscle potassium content or extracellular pH. Their data and the aforementioned renal metabolic changes, however, might be explained by local changes in cell potassium concentration, alteration in the transcellular potassium or hydrogen ion ratios, or other yet unexplored possibilities. It seems logical to conclude that intracellular pH changes induced by alterations in potassium metabolism do indeed affect cellular metabolism, but the present methods available for measuring cell pH do not allow for a precise definition of these interrelationships or the mechanisms by which they occur.

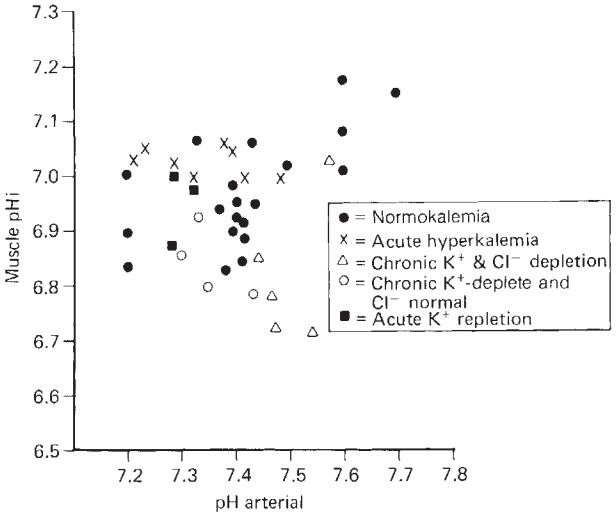

Fig. 2. *The comparison of arterial pH and skeletal muscle pH_i, determined by the DMO method for in vivo rat and dog experiments at all levels of plasma potassium.* Data are from References 22, 32, 33, 45–48, 63.

Summary

Recent work has clarified some of the complex interrelationships between cell pH and potassium. These studies have been limited by the techniques available for accurately measuring cell pH. At present it is obvious that intracellular pH is a major regulator of the cellular potassium concentration, but the precise relationship between these two is still uncertain. It has become increasingly clear, however, that no simple relationship exists between the intracellular to extracellular hydrogen ion and potassium ion ratios. Many experiments do demonstrate that the extracellular metabolic alkalosis of potassium depletion is accompanied by a decrease in skeletal muscle pH in rat, rabbit, and probably dog. The response of cardiac and renal tubular cell pH to

potassium depletion is less clear, although most evidence indicates that there is also a reduction in the pH of these tissues. This effect on cell pH appears to be independent of chloride. By contrast, hyperkalemia seems to raise muscle cell pH at the same time it induces an extracellular metabolic acidosis. The metabolic and physiologic consequences of potassium-induced alterations in cell pH have yet to be fully elucidated.

Reprint requests to Dr. S. Adler, Department of Medicine, University of Pittsburg School of Medicine, Pittsburgh, Pennsylvania 15213, U.S.A.

References

1. COOKE RE, SEGAR WE, CHEEK DB, COLVILLE FE, DARROW DC: The extrarenal correction of alkalosis associated with potassium deficiency. *J Clin Invest* 31:798–805, 1952
2. BLACK DAK, MILNE MD: Experimental potassium depletion in man. *Clin Sci* 11:397–408, 1952.
3. HUDSON JB, RELMAN AS; Effects of potassium and rubidium on muscle cell bicarbonate. *Am J Physiol* 203:209–214, 1962
4. ADDANKI SFD, CAHILL FD, SOTOS JF: Passive transport of 5, 5-dimethyl-2, 4-oxazolidinedione into beef heart mitochondria. *Science* 155:1678–1679, 1967
5. DANIELLI JF: On the pH at the surface of ovalbumen molecules and protein error with indicators. *Biochem J* 35:470–478, 1941
6. ADLER S: The simultaneous determination of muscle cell pH using a weak acid and weak base. *J Clin Invest* 51–251–265, 1972
7. CARTER NW: Intracellular pH. *Kidney Int* 1:341-346, 1972
8. CHANCE B, MELA L: Intramitochondrial pH changes in cation accumulation. *Proc Natl Acad Sci US* 55:1243–1251, 1966
9. HERBST M, PIONTEK P: Über den verlauf des intracellulären pH: Wertes des skeletmuskeln während der kontraktion. *Pfluegers Arch* 355:213–223, 1972
10. MOON RB, RICHARDS JH: Determination of intracellular pH by ^{31}P magnetic resonance. *J Biol Chem* 248:7276–7278, 1973
11. COHEN RD, ILES RA: Intracellular pH: Measurement, control, and metabolic interrelationships. *CRC Crit Rev Clin Lab Sciences* 6:101–143, 1975
12. CALDWELL PC: Studies on the internal pH of large muscle and nerve fibers. *J Physiol* 142:22–62, 1958
13. BICHER HI, OHKI S: Intracellular pH electrode experiments on the giant squid axon. *Biochim Biophys Acta* 255:900–904, 1972
14. KHURI RN, AGULIAN SK, BOGHARIAN KK: Intracellular bicarbonate of skeletal muscle under different metabolic states. *Am J Physiol* 230:228–232, 1976
15. WIGGINS PM: Intracellular pH of deep and superficial muscle fibers. *J Theor Biol* 42:113–117, 1973
16. CALDWELL PC: Intracellular pH, in International Review of Cystology, edited by BOURNE GH, DANIELLI JF, New York, Academic, vol. 5, 1956, p. 229–277
17. WADDELL WJ, BATES RG: Intracellular pH. *Physiol Rev* 49:285–329, 1969
18. FENN WO, COBB DM: The potassium equilibrium in muscle. *J Gen Physiol* 17:629–645, 1934
19. SCRIBNER BH, FREMONT-SMITH K, BURNELL JM: The effect of acute respiratory acidosis on the internal equilibrium of potassium. *J Clin Invest* 34:1276–1285, 1955
20. BURNELL JM, VILLAMIL MF, UYENO BT, SCRIBNER BH: The effect in humans of extracellular pH change on the relationship between serum potassium concentration and intracellular potassium. *J Clin Invest* 35:935–939, 1956
21. LIEBMAN J, EDELMAN IS: Interrelationship of plasma potassium concentration, plasma sodium concentration, arterial pH, and total exchangeable potassium. *J Clin Invest* 38:2176–2188, 1959
22. BROWN EB JR, GOOTT B: Intracellular hydrogen ion changes and potassium movement. *Am J Physiol* 204:765–770, 1963
23. YOUNG WG, SEALY WC, HARRIS JS: Role of intracellular and extracellular electrolytes in cardiac arrhythmias produced by prolonged hypercapnia. *Surgery* 36:636–646, 1954
24. LADE R, BROWN EB JR: Movement of potassium between muscle and blood in response to respiratory acidosis. *Am J Physiol* 204:761–764, 1963
25. LAI YL, ATTERBY BA, BROWN EB: Mechanism of cardiac muscle adjustment to hypercapnia. *Respir Physiol* 19:123–129, 1973
26. BROWN EB JR, MOWLEM A: Potassium loss from the heart in the immediate post hypercapnic period. *Am J Physiol* 198:962–964, 1960
27. KIM WB, BROWN EB: Potassium transfer with constant extracellular pH. *J Lab Clin Med* 71:678–685, 1968
28. SCRIBNER BH, FREMONT-SMITH K, BURNELL JM: The effect of acute respiratory acidosis on the internal equilibrium of potassium. *J Clin Invest* 34:1276–1285, 1955
29. GEIBISCH G, BERGER L, PITTS RF: The extrarenal response to acute acid-base disturbances of respiratory origin. *J Clin Invest* 34:231–245, 1955
30. KEATING RE, WEICHSELBAUM TE, ALANIS M, MARGRAF HW, ELMAN R: The momement of potassium during experimental acidosis and alkalosis in the nephrectomized dog. *Surg Gynecol Obstet* 96:323–330, 1953
31. TOBIN R: Varying role of extracellular electrolytes in metabolic acidosis and alkalosis. *Am J Physiol* 195:685–692, 1958
32. MAKOFF DL, DA SILVA JA, ROSENBAUM BJ: On the mechanism of hyperkalemia due to hyperosmotic expansion with saline or mannitol. *Clin Sci* 41:383–393, 1971
33. IRVINE ROH, DOW J: Muscle cell pH and potassium movement in metabolic acidosis. *Metabol Clin Exper* 17:563–570, 1968
34. ADLER S: Cellular pH in potassium depletion. *J Lab Clin Med* 78:1013, 1971
35. FRALEY DS, ADLER S: Isohydric regulation of plasma potassium by bicarbonate in the rat. *Kidney Int* 9:333–343, 1976
36. GULYASSY PF, VAN YPERSELE DE STRIHOU C, SCHWARTZ WB: On the mechanism of nitrate-induced alkalosis: The possible role of selective chloride depletion in acid-base regulation. *J Clin Invest* 41:1850–1862, 1962
37. KASSIRER JP, SCHWARTZ WB: The response of normal man to selective depletion of hydrochloric acid: Factors in the genesis of persistent gastric alkalosis. *Am J Med* 40:10–18, 1966
38. ADLER S, ZETT B, ANDERSON B: The effect of acute potassium depletion on muscle cell pH *in vitro*. *Kidney Int* 2:159–163, 1972
39. COHEN JJ: Correction of metabolic alkalosis by the kidney after isometric expansion of extracellular fluid. *J Clin Invest* 47:1181–1192, 1968
40. ATKINS EL, SCHWARTZ WB: Factors governing correction of the alkalosis associated with potassium deficiency: The critical role of chloride in the recovery process. *J Clin Invest* 41:218–229, 1962
41. KASSIRER JP, SCHWARTZ WB: Correction of metabolic alkalosis in man without repair of potassium deficiency: A re-

evaluation of the role of potassium. *Am J Med* 40:19–26, 1966
42. ECKEL RE, NORRIS JEC, POPE EC: Basic amino acids as intracellular cations in potassium deficiency. *Am J Physiol* 193:644–652, 1958
43. ECKEL RE, BOTSCHNER AW, WOOD DH: The pH of K-deficient muscle. *Am J Physiol* 196:811–818, 1959
44. GARDNER LI, MACLACHLAN EA, BERMAN H: Effect of potassium deficiency on carbon dioxide, cation, and phosphate content of muscle. *J Gen Physiol* 36:153–160, 1952
45. WILSON AF, SIMMONS DH: Relationships between potassium, chloride, intracellular and extracellular pH in dogs. *Clin Sci* 39:731–745, 1970
46. SANSLONE WR, MUNTWYLER E: Intracellular pH changes associated with protein and potassium deficiency. *Metabolism* 17:1084–1093, 1968
47. GRANTHAM JJ, SCHLOERB PR: Interrelationship of potassium and hydrogen ion gradients in metabolic alkalosis. *Am J Physiol* 208:1114–1118, 1965
48. BURNELL JM, DAWBORN JK: Acid-base parameters in potassium depletion in the dog. *Am J Physiol* 218:1583–1589, 1970
49. CAMERON JR, HALL RJC: The effect of dietary K^+ depletion and subsequent repletion on intracellular K^+ concentration and pH of cardiac and skeletal muscle in rabbits. *J Physiol* 251:70P–71P, 1975
50. MILLER RB, TYSON I, RELMAN AS: pH of isolated resting skeletal muscle and its relation to potassium content. *Am J Physiol* 204:1048–1054, 1963
51. SANSLONE WR, MUNTWYLER E: Effect of altered plasma pCO_2 on intracellular pH during potassium deficiency. *Proc Soc Exp Biol Med* 126:750–754, 1967
52. SCHLOERB PR, BLACKBURN GL, GRANTHAM JJ: Carbon dioxide dissociation curve in potassium depletion. *Am J Physiol* 212:953–956, 1967
53. IRVINE ROH, SAUNDERS SJ, MILNE MD, CRAWFORD MA: Gradients of potassium and hydrogen ion in potassium deficient voluntary muscle. *Clin Sci* 20:1–18, 1961
54. STRUYVENBERG A, MORRISON RB, RELMAN AS: Acid-base behavior of separated canine renal tubule cells. *Am J Physiol* 214:1155–1162, 1968
55. ADLER S, ZETT B, ANDERSON B: Renal citrate in the potassium-deficient rat: Role of potassium and chloride ions. *J Lab Clin Med* 84:307–316, 1974
56. BURNELL JM, TEUBNER EJ, SIMPSON DP: Metabolic acidosis accompanying potassium deprivation. *Am J Physiol* 227:329–333, 1974
57. MCQUARRIE I, JOHNSON RM, ZEIGLER MR: Plasma electrolyte disturbance in patient with hypercortical-adrenal syndrome contrasted with that found in Addison's disease. *Endocrinology* 21:762–772, 1937
58. WILLSON DM, POWER MH, KEPLER EJ: Alkalosis and low plasma potassium in a case of Cushing's syndrome: Metabolic study. *J Clin Invest* 19:701–707, 1940
59. LUKE RG, LEVITIN H: Impaired renal conservation of chloride and the acid-base changes associated with potassium depletion in the rat. *Clin Sci* 32:511–526, 1967
60. GARELLA S, CHAZAN JA, COHEN JJ: Saline-resistant metabolic alkalosis or "chloride wasting nephropathy." *Ann Intern Med* 73:31–38, 1970
61. SELDIN DW, WELT LG, CORT JH: The role of sodium salts and adrenal steroids in the production of hypokalemic alkalosis. *Yale J Biol Med* 29:229–247, 1956
62. RELMAN AS, ROY AM, SCHWARTZ WB: The acidifying effect of rubidium in normal and potassium-deficient alkalotic rats. *J Clin Invest* 34:538–544, 1955
63. HUDSON JB, RELMAN AS: Effects of potassium and rubidium on muscle cell bicarbonate. *Am J Physiol* 203:209–214, 1962
64. DUA SL, MAKOFF DL, KUYT F, DASILVA JA, MASSRY SG: Effect of potassium chloride infusion on the intracellular and extracellular acid-base balance in the dog. *Kidney Int* 8:473, 1975
65. PAILLARD M: Direct intracellular pH measurement in rat and crab muscle. *J Physiol (London)* 223:297–319, 1972
66. HUTH EI, SQUIRES RD, ELKINTON JR: Experimental potassium depletion in normal human subjects: II. Renal and hormonal factors in the development of extracellular alkalosis during depletion. *J Clin Invest* 38:1149–1165, 1959
67. FOURMAN P, ROBINSON JR: Diminished urinary excretion of citrate during deficiencies of potassium in man. *Lancet* 265:656–657, 1953
68. ADLER S, ANDERSON B, ZETT B: Regulation of citrate metabolism by cell pH in potassium-depleted rat diaphragm. *Kidney Int* 6:92–98, 1974