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in Membranes and Transport

VOLUME 28

POTASSIUM TRANSPORT: PHYSIOLOGY AND PATHOPHYSIOLOGY

Guest Editor
Gerhard Giebisch

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Potassium Transport: Physiology and Pathophysiology

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VOLUME 28

Potassium Transport: Physiology and Pathophysiology

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Preface

On April 12–13, 1985, a symposium, sponsored by the Departments of Physiology and Medicine, was held at the Yale University School of Medicine to honor Robert W. Berliner on the occasion of his retirement. Given a choice of selecting a topic, he chose potassium transport. This volume is based largely on the contributions to this symposium. They focus mainly, but not exclusively, on the role of potassium in epithelial transport, especially that occurring in the kidney and the colon. Several chapters dealing with the mechanisms controlling potassium distribution and transport in nonepithelial tissues are also included. An attempt was made to stress cellular transport events in terms of an analysis of potassium translocation across individual cell membranes and intercellular pathways to extend the analysis of potassium transport beyond a descriptive "black-box" approach.

The first part of this volume is concerned with basic mechanisms of potassium transport in epithelia and across cell membranes. Discussions of different modes of potassium movement by the sodium–potassium pump, by cotransport with sodium and chloride, and by passive electrodiffusion are included. Present in both symmetrical and polar tissues, these three types of distinct ion translocation also constitute the building blocks of potassium translocation in epithelia. Depending on the specific spatial distribution in the apical and basolateral membrane of epithelial cells, the very same transport mechanisms determine direction and magnitude of vectorial potassium movement.

The second part contains a careful analysis of renal potassium handling. Following chapters that deal with the definition of the nephron sites of potassium transport, several cell models at the strategic sites of potassium movement along the nephron are defined. Contributions on hormonal and acid–base factors known to exert profound effects on renal potassium movement follow. Finally, the functional and morphological correlates of renal potassium adaptation are analyzed.

The third part extends the analysis of renal potassium transport to pathophysiological derangements of potassium transport. The events underlying hypokalemia and hyperkalemic states are discussed, including a careful evaluation of the effects of diuretics on the renal excretion of potassium.

In the last part of the volume, extrarenal aspects of potassium metabo-

lism are analyzed. A detailed discussion of the complex interplay of several factors regulating the internal potassium balance and of potassium transport in the colon is included.

The choice of the topic was a very appropriate one since Bob Berliner and his associates Tom Kennedy and Jack Orloff—first at Goldwater Memorial Hospital and then at the National Heart Institute—were among the first to study in depth the renal handling of potassium. Dr. Berliner's team provided the first set of extensive experimental results that described the main features of potassium excretion by the kidney. They defined the key factors regulating potassium metabolism and, with bold intuition, provided one of the earliest cell models of epithelial potassium transport. Thus, new concepts and new ways of reasoning were introduced into the field of renal physiology. To cite but one, the demonstration that ions such as potassium were secreted into the urine in exchange for a counterion (sodium) marked a significant advance and has stimulated many transport studies extending beyond the epithelium of renal tubules.

Renal research owes much to these early renal transport studies in the 1950s. They provided a firm foundation for later work on single tubules and single tubule cells, and they made it possible to evaluate the early concepts directly. Thus, everyone who studies epithelial potassium transport builds from the foundation established by the work of Bob Berliner and his associates.

In addition to Bob Berliner's solid achievements as one of the world's leading renal physiologists, the biomedical community is also grateful for the eminent role he has played in stimulating critical research. While at the National Institutes of Health—where he started as head of the Laboratory of Kidney and Electrolyte Metabolism in 1950 and rose to be Deputy Director of Science in 1968—he attracted a group of outstanding associates. They carried on his tradition of excellence and have helped make the field of nephrology an exciting and respected one. Later, during his deanship at Yale Medical School, and also at the present time, Bob Berliner remains active in and a critical scholar of renal research. He is always willing to listen to a problem, and one never leaves him without having been helped and having gained new insights.

It is appropriate to quote from the award ceremony at Yale where Bob Berliner received an honorary Degree of Science in 1973 "For outstanding scientific contributions, and in appreciation of your role as the nation's leading statesman in biomedical science, Yale takes pride in conferring upon you the degree of Doctor of Science."

I join with all of those who were present at the symposium in extending our gratitude and indebtedness to Bob Berliner.

We would like to recognize gratefully the support of the following

sponsors of the symposium: Abbott Laboratories, The American Heart Association, Burroughs Wellcome Fund, Ciba Geigy Pharmaceutical Corporation, Hoechst-Roussell Pharmaceuticals, Inc., Merck, Sharp & Dohme Research Laboratories, Miles Laboratories, Inc., The Upjohn Company, and Wyeth Laboratories. We also wish to particularly recognize the Sandoz Research Institute for its very generous support.

We thank the Office of Public Information of Yale University for the photo of Dr. Berliner.

GERHARD GIEBISCH

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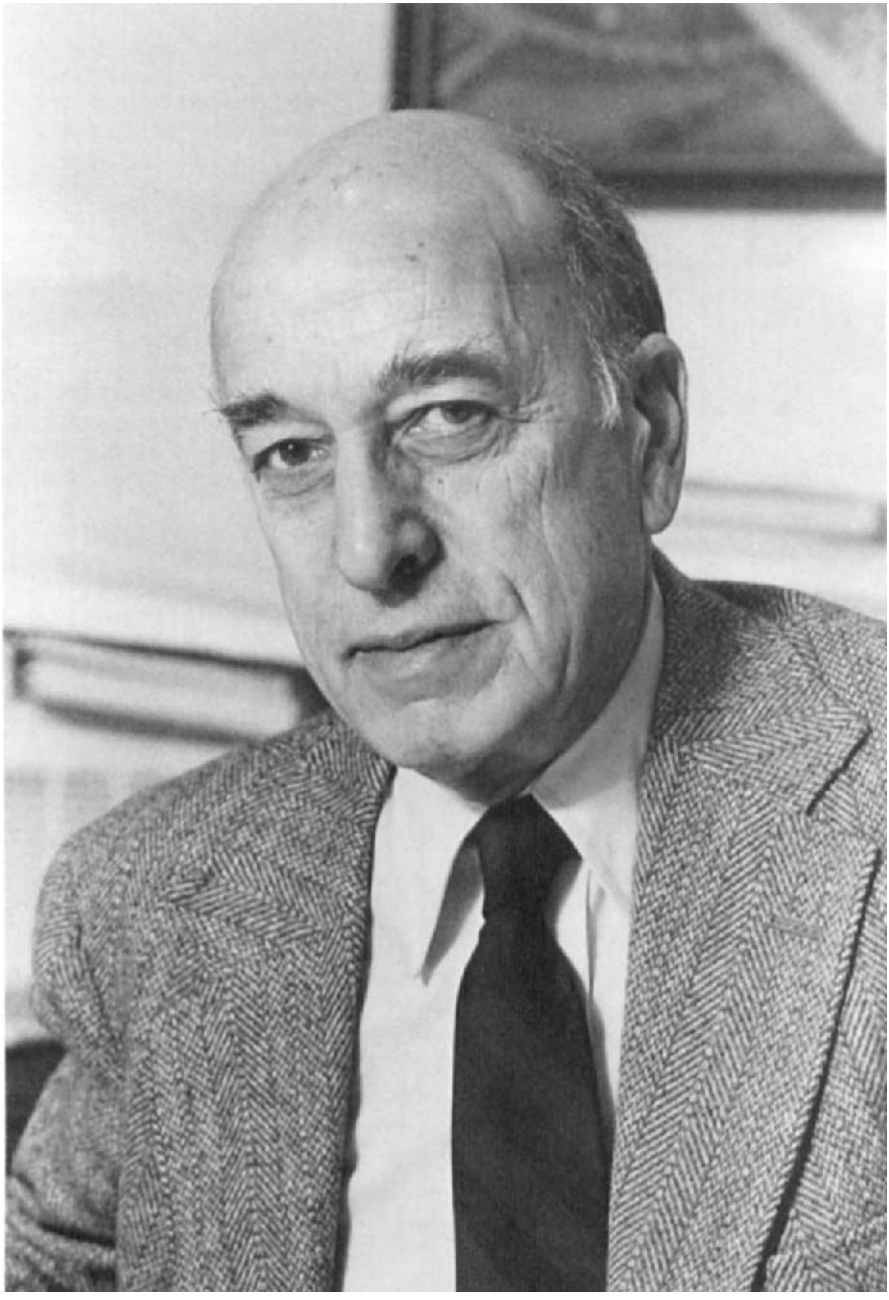
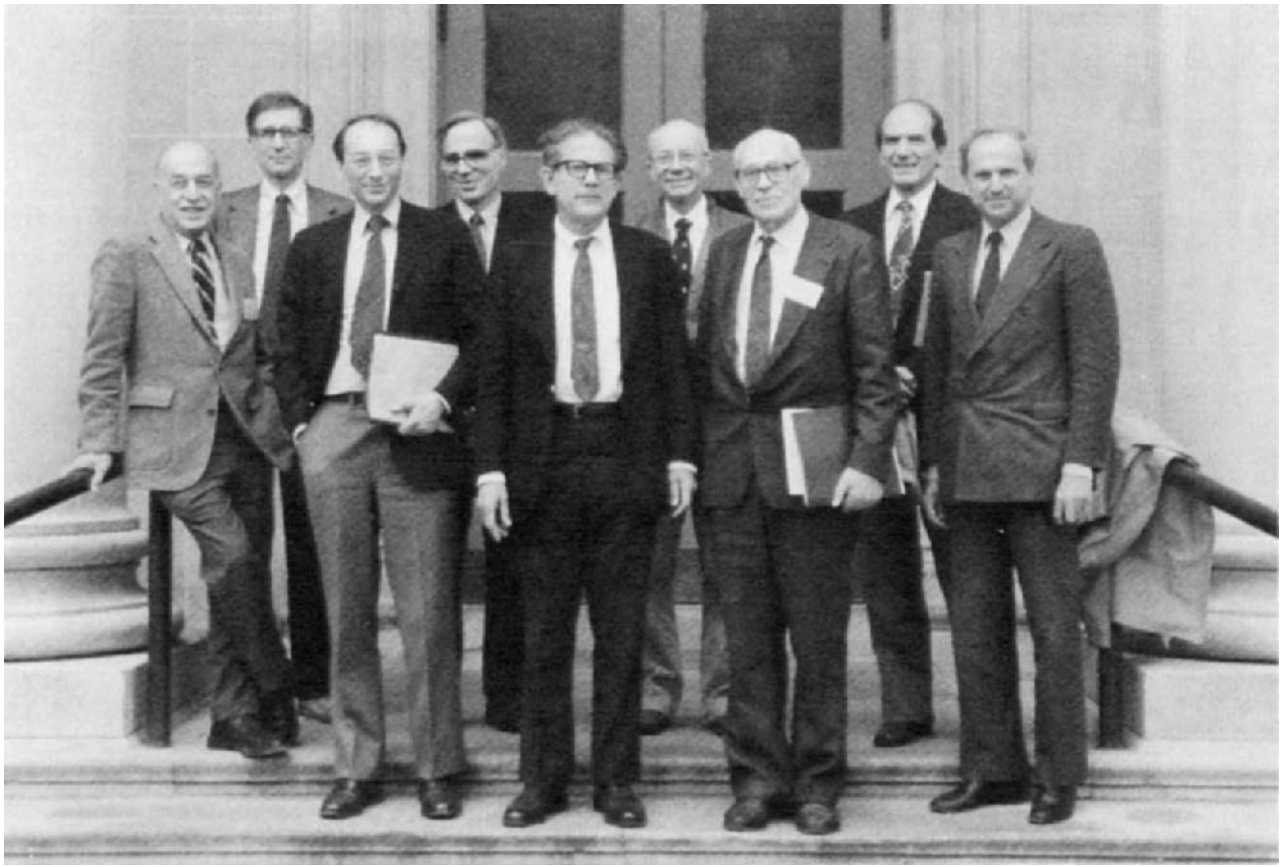


Dr. Robert W. Berliner
Dean
Yale University School of Medicine
Professor of Physiology and Medicine



DR. BERLINER AND FRIENDS
Back row, from left: J. F. Hoffman, F. S. Wright, G. Whittembury, and G. Malnic
Front row, from left: R. W. Berliner, G. Giebisch, J. Orloff, H. H. Ussing, and E. L. Boulpaep

Part I

Cell Mechanism of Potassium Transport

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Chapter 1

Role of Potassium in Epithelial Transport Illustrated by Experiments on Frog Skin Epithelium

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I. AN UPDATED MODEL OF THE EPITHELIUM

Our early work on the frog skin demonstrated that the epithelium performed active inward transport of sodium and that chloride followed due to the electric potential developed by the sodium transport. Potassium seemed not to be transported. We soon found, however, that potassium played a very important role in the process, but that it was recycled across the basolateral membrane of the cells, while the apical cell membrane was virtually tight to potassium (Koefoed-Johnsen and Ussing, 1958).

A. Main Na^+/Transport via Syncytium of Principal Cells

In the years that have passed since we proposed the two-membrane theory, it has served well to explain many of the transport properties of especially tight epithelia. In recent years, the simple model has been supplemented by the discovery of new features. They do not invalidate the simple model, but explain apparent discrepancies between experiment and theory. One important step forward was the realization (Ussing and Windhager, 1964; Farquahar and Palade, 1964; Rick *et al.*, 1978) that the vast majority of epithelium cells form a functional three-dimensional syncytium, so that the whole acts as one cell, with the properties postulated in the two-membrane theory. Another important finding was that the outward facing membrane of the outermost layer of syncytium cells has sodium channels, but that this membrane is always tight to chloride. The

chloride permeability of the epithelium is localized to the so-called mitochondria-rich cells which make up only a small fraction of the epithelial volume. The organization of the main transport mechanisms of the epithelium is shown diagrammatically in Fig. 1. The syncytium of principal cells is shown as one large cell, and the mitochondria-rich cell is shown as a smaller one. The large "cell" performs the bulk of sodium transport. Its apical membrane has specific sodium channels which will conduct only sodium and lithium. A dashed arrow indicates

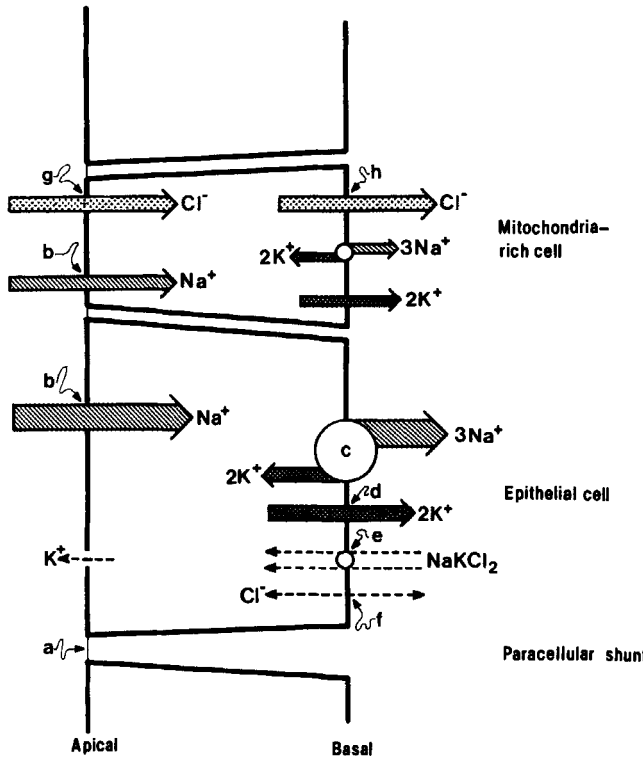

FIG. 1. Organization of the main electrolyte transport pathways in frog skin epithelium. The syncytium of principal cells is shown as one large cell and the mitochondria-rich cell as a smaller one. The large cell performs most of the sodium transport. Its apical membrane has specific sodium channels. A broken arrow shows that there may also be a small number of potassium channels. The basolateral membrane has potassium channels and the sodium/potassium pump. Shown with broken lines are chloride channels and a cotransport of Na + K + 2 Cl. These two types of mechanisms are normally working slowly or not at all. The mitochondria-rich cells have chloride channels in both membranes and represent the only important transcellular chloride pathway. Those in the apical membrane are potential gated. These cells also have sodium channels in the apical and potassium channels as well as ion pumps in the basolateral membrane. Also shown is a paracellular shunt, which, however, is normally of secondary importance.

that there is also a small number of potassium channels of the type found in the basolateral membrane. More potassium channels may be activated or inserted under special conditions. Thus, closure of the basolateral channels with Ba^{2+} may augment the apical efflux of potassium, but this exit path can be inhibited by apical application of Ba^{2+} (Nielsen, 1985). Increased apical leakiness to potassium can also be elicited, keeping the frog in high K solution for a number of days before the experiment.

The basolateral membrane has potassium channels and the sodium/potassium pump. The coupling ratio for the two ions seems always to be 3/2. Convincing evidence for this statement has been provided by Nielsen (1979), who showed that the short-circuit current of isolated epithelia dropped memomentarily to exactly one-third when the potassium channels of the basolateral membrane were closed by addition of Ba^{2+} to the inside bath. This is what one would predict under the assumption that the normal short-circuit current is composed of one "electrogenic" sodium ion plus two potassium ions which are recycled via the potassium channels.

In addition to the ion pump and the potassium channels, these cells also possess a chloride channel and a cotransport mechanism which seems to be of the (Na + K + 2 Cl) type. Both mechanisms are shown with dashed arrows, indicating that they are normally insignificant or nonfunctioning. We shall return to these mechanisms in the following.

B. Mitochondria-Rich Cells as Main Chloride Pathway

In contrast to the syncytium cells, the mitochondria-rich cells have chloride channels in the apical as well as in the basolateral membrane. These cells also have sodium channels in the apical and potassium channels as well as an ion pump in the basolateral membrane; however, whereas the mitochondria-rich cells account for virtually the total chloride conductance of the epithelium, their sodium transport is a minor fraction of the total. The chloride transport has been known for years to be passive, obeying the flux ratio equation. The evidence for a localization of the Cl conductance to the mitochondria-rich cells until quite recently was indirect [for references, see Voute and Meier (1978), Kristensen (1981), and Ussing (1982)].

Quite recently, using toad skin in which the sodium channels had been closed by amiloride, Scheffey and Katz (1985) were able to show that electric current from a microelectrode showed steep maximum when the electrode approached a mitochondria-rich cell. Also, Spring and Ussing and Foskett and Ussing (both in preparation) have shown that the mitochondria-rich cells swell when chloride replaces a nonpenetrating anion in the outside bath, whereas the syncytium cells show absolutely no volume response.

C. Insignificant Apical Potassium Leak

It should be emphasized that the scanty potassium conductance of the apical membrane indicated for the syncytium cells might, in fact, reside in the apical membrane of the mitochondria-rich cells or in both cell types.

D. Insignificant Paracellular Shunt

Figure 1 also indicates a paracellular shunt path. It is normally insignificant for potassium as well as for sodium and chloride. If, however, the outside medium is made hypertonic relative to that of the inside, the transepithelial resistance drops dramatically due to opening of the tight seals, and all ions as well as medium-sized nonelectrolytes such as sucrose can pass (Ussing and Windhager, 1964).

II. TRANSPORT MECHANISMS REVEALED BY EPITHELIAL VOLUME RESPONSES

A. Earlier Findings

In the following, we shall discuss in more detail the two "newcomers" in the model of the syncytium cells, namely, first, the basolateral cotransport of Na^+, K^+, and chloride, and second, the variable chloride channel. These mechanisms, in collaboration with the sodium/potassium pump and the potassium channels, are involved in the volume regulation and determine the K^+ content of the epithelium. I find it appropriate on this occasion to report on my recent research in this field, because I did the very first crucial observations when visiting Dr. Berliner's laboratory in the Heart Institute of The National Institutes of Health in 1957. After all these years, it is finally possible to explain the observations in a satisfactory way. The object of my visit to Bethesda was to develop a method for measuring the thickness of the epithelium while the skin was mounted in a chamber where inside and outside medium could be changed independently.

It had become desirable to make such a study because it seemed possible to test some of the consequences of the two-membrane theory (Koefoed-Johnsen and Ussing, 1958) by following the volume responses of the epithelium when the outside and inside media were varied in a systematic way. The first study was completed after my return to Copenhagen in collaboration with Dr. Enid MacRobbie (MacRobbie and Ussing, 1961), who spent the following year with us as a postdoctoral fellow. Some of the findings were clear-cut and uncontroversial. Thus, we found that the water permeability of the outward-facing side of the

epithelium was very low compared to that of the inward-facing membrane. Changes in the osmolarity of the outside medium had virtually no effect on the epithelium volume, whereas fast changes occurred when the osmolarity of the inside medium was altered. In fact, if the inside medium was sulfate Ringer (sulfate replacing chloride), the epithelium acted as an ideal osmometer over a wide range of osmolarities. However, if the inside medium was ordinary Ringer, the epithelium behaved quite differently: In half Ringer, it swelled rapidly, but then lost most of the volume gained. When the inside medium was again made full-strength Ringer, the epithelium shrank, but regained volume so that the epithelium ended having its original volume. We were able to show that the volume changes were due to loss, gain, respectively, of potassium and chloride ions to and from the inside medium. We concluded that either K^+ or Cl^- (or both) must be present in the cells at a concentration far higher than that required for electrochemical equilibrium with the inside bathing medium (see also Dörge *et al.*, 1981), and that cell swelling increased the permeability to both ions so that Donnan distribution of the two ionic species would be established across the inward-facing membrane. All recent measurements of the intracellular potential indicate that K^+ is not far from electrochemical equilibrium with the inside solution. It is chloride that is present in the epithelium at a concentration far above that required for equilibrium with the inside bath (see, for instance, Nagel, 1976; Hellman *et al.*, 1979; Nielsen, 1985). This means that there must be an energy-requiring process which maintains chloride at its high cellular concentration and which restores cell chloride to its normal concentration when the epithelium recovers in normal Ringer after exposure to dilute inside solutions. It now seems clear that the recovery is due to a cotransport $NaKCl_2$, located in the basolateral membrane of the syncytium cells (Ussing, 1982, 1985). The chloride uptake can be considered as secondary active transport: There is a downhill gradient for the entity of one Na^+, one K^+, and two Cl^- from the inside medium to the cytoplasm, since the product of the activities of the four ions is higher in the medium than in the cells. The sodium/potassium pump maintains the cellular Na^+ concentration at a low value, and therefore Cl^- and K^+ will accumulate in the cells. If, futhermore, the chloride channels are closed, the accumulation of K^+ and Cl^- should continue until the cotransport is turned off or a steady state is established.

B. Evidence for Basolateral Cotransport of $Na^+ \pm K^+ \pm 2\,Cl^-$

Some of the properties of the volume regulation mechanisms are illustrated in Fig. 2. The curve with experimental points indicated as dots gives the epithelial thickness in microns, whereas the continuous (lower) line is the potential dif-

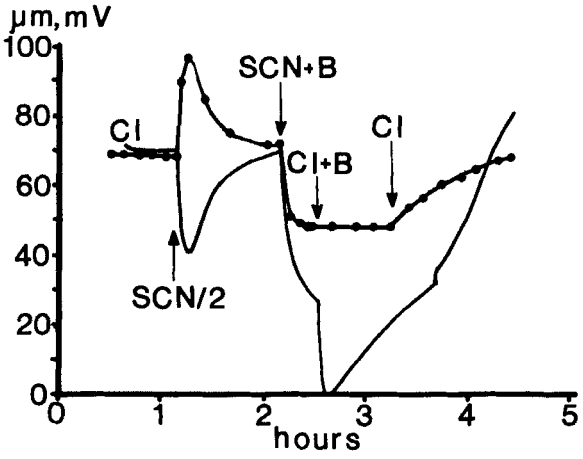

FIG. 2. Main properties of the volume regulation mechanisms of the epithelium (i.e., the syncytium cells). Ordinate is the epithelial thickness in microns [curve with experimental points and transepithelial potential difference (smooth curve)]. Abscissa: Time in hours.

At zero time, the inside medium is ordinary Ringer. The outside medium is 1/20 Ringer throughout. At the first arrow (SCN/2), the inside medium is changed to half-strength thiocyanate Ringer. At the second arrow (SCN + B), the medium is made full thiocyanate Ringer, with bumetanide added to a concentration of 50 µmol/liter. At the fourth arrow (Cl), the inside medium is changed back to ordinary Ringer.

ference. The outside medium is 1/20 frog Ringer throughout. (The use of 1/20 Ringer as outside medium has the advantage that it has a sodium concentration not very far from that of the epithelial cells. Therefore, the sodium diffusion potential across the apical membrane is small and the transmural potential difference consequently is a rough estimate of the basolateral potential difference.) The inside medium is initially Ringer. At the first arrow, this medium is replaced by thiocyanate Ringer (thiocyanate substituting for Cl^-). The epithelium swells rapidly from 70 to 97 µm. Simultaneously, the potential difference drops from a value close to the potassium equilibrium potential to a value between the latter and the chloride potential. In other words, the chloride channels of the basolateral membrane open *pari passu* with the swelling, and consequently (since the potassium channels of that membrane are always open), the cells lose KCl to the inside bath. Within 1 hr the cells lose all their chloride, and simultaneously, the potential reverts to its original value. The cell volume at this time must be double the chloride-free one. Now, at the second arrow, full thiocyanate Ringer is applied as inside bath, and the epithelium shrinks to what must be half the double chloride-free volume. The drop in thickness thus gives the chloride-free volume, and the difference between the new steady state and the original level (before the first arrow) gives the chloride space. This is a clear demonstration that nearly

half the osmotic volume of these cells is represented by KCl. At the third arrow, the inside bath is made ordinary (chloride) Ringer, with bumetanide added to a concentration of 50 μmol/liter. It is seen that the volume remains constant for 1 hr, indicating that the recovery of volume is inhibited by bumetanide. Finally, at the fourth arrow, the inside bath is made ordinary Ringer without bumetanide, and the volume reverts to its original value.

The potential difference responses related to the shrinkage and recovery phases probably have to do with closure of the sodium channels when the cells shrink and reopen during volume recovery. It is conceivable that the slow potential increase during the bumetanide period is due to a very slow swelling.

The experiment shows that the cotransport responsible for volume recovery requires chloride as well as sodium. It is known that lithium can, but that choline and arginine cannot, replace sodium (Ussing, 1982, 1985).

When the participation of a cotransport in the volume regulation was proposed (Ussing, 1982; see also Ferreira and Ferreira, 1981), it was left undecided whether it involved only Na^+ and Cl^- or whether it also involved K^+. However, the latter possibility is strongly supported by recent experiments (Ussing, 1985). The requirement of potassium for recovery of volume after loss of KCl (and volume) is illustrated in Fig. 3. The initial steps of the experiment are the same as in Fig. 2. At the first arrow, Ringer of the inside bath is replaced by half thiocyanate Ringer. After swelling, the epithelium loses volume (and KCl), and

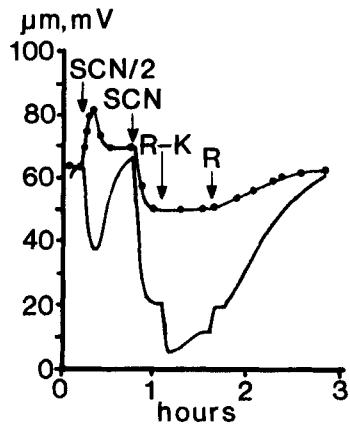

FIG. 3. Requirement of potassium in the inside bath for recovery of volume after loss of K and Cl during hypoosmotic chock. Ordinate and abscissa as in Fig. 2. Outside bath 1/20 Ringer throughout. Inside bath originally Ringer. At the first arrow (SCN/2), the inside bath is changed to half thiocyanate Ringer. At the second arrow (SCN), the inside medium is made full thiocyanate Ringer. At the third arrow (R–K), the inside medium is made ordinary Ringer without potassium. At the fourth arrow (R), the inside medium is changed to ordinary Ringer, with the usual content of potassium (1.8 mmol/liter).

a steady state with chloride-free cells is achieved. At the second arrow, full thiocyanate Ringer replaces the dilute solution and the cells shrink to a new steady state. At the third arrow, the inside bath is made potassium-free Ringer. For 30 min there is no change in thickness, but as soon as (at the fourth arrow) ordinary Ringer (with 1.8 mmol K^+ per liter) is applied, the epithelium starts swelling, and within 1 hr the thickness is exactly what it was at the beginning of the experiment. Thus, potassium as well as sodium and chloride are necessary for the recovery process. Note that the potential difference responses are very similar to the one seen in Fig. 2.

The two foregoing experiments have demonstrated that the osmotic swelling opens the chloride channels so that the epithelium loses KCl and volume. That the cation lost (and regained) is indeed potassium has been shown by chemical analysis (MacRobbie and Ussing, 1961; Ussing, 1982). The normal steady-state volume requires that chloride is maintained at a cellular concentration much higher than required for electrochemical equilibrium with the inside bath (compare Nagel, 1976). It stands to reason that the process responsible for the normal high chloride concentration is the very cotransport which has been illustrated in Figs. 2 and 3. The recovery of cellular volume after KCl loss is just as effective with distilled water outside as with Ringer. Thus, only the basolateral membrane is involved. We shall now consider the question whether the cotransport is only activated when required or whether the normal cell volume (and chloride concentration) is a "pump leak" equilibrium. Now that we know that the cotransport is inhibited by bumetanide, it may be of interest to study the effect of that drug on the normal epithelial volume. The experiment shown in Fig. 4 illustrates this point. At the first arrow, Ringer + bumetanide replaces Ringer. It is seen that in the course of 1 hr, there is no change in volume and no potential response. At the second arrow, the bath is made nitrate Ringer (nitrate replaces Cl). This has no effect on the potential difference, but after 30 min, a slow shrinkage sets in, and after another hour a new quasi-steady state is established. It is clear that the normal turnover rate of chloride due to leakage and cotransport must be very slow indeed. Over very long periods of time, the replacement of chloride by ions which do not participate in the cotransport does lead to loss of volume. The process is very slow for nitrate and thiocyanate, but seems to be faster for sulfate (MacRobbie and Ussing, 1961; Ferriera and Ferriera, 1981). A very slow shrinkage is also seen when the inside bath is K^+-free Ringer (Ussing, 1965). If sodium is replaced by choline or arginine, it is hardly possible to demonstrate any shrinkage within 1 hr. The observations lead to the conclusion that at its normal volume, the epithelium has a very low permeability to chloride, and the cotransport must also be going at a very slow rate. It has been pointed out recently (Ussing, 1985) that if the transported entity is $NaKCl_2$, the normal cells may not be far from equilibrium.

Returning now to Fig. 4, we notice that when, at the third arrow, the inside

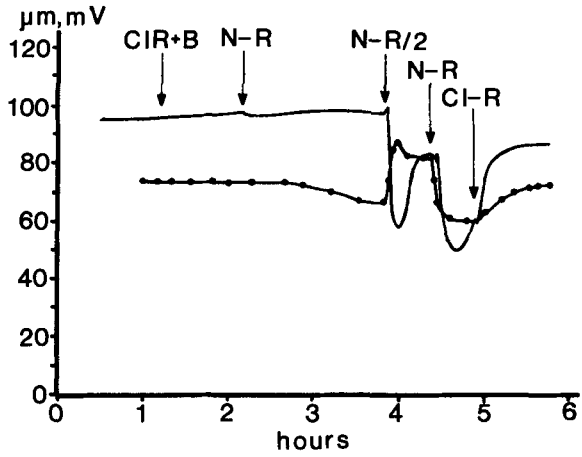

FIG. 4. The normal rate of loss and recovery of chloride is shown as very slow. Ordinate and abscissa as in Fig. 2. Outside medium 1/20 Ringer throughout. Inside bath initially Ringer. At the first arrow (ClR + B), the inside medium is changed to Ringer, with bumetanide added to a concentration of 50 µmol/liter. At the second arrow (N–R), the inside medium is changed to nitrate Ringer. At the third arrow (N–R/2), the inside medium is made half-strength nitrate Ringer. At the fourth arrow (N–R), the medium is again full nitrate Ringer, and finally, at the fifth arrow (Cl–R), the medium is ordinary Ringer.

medium is made half nitrate Ringer, the swelling is followed by a relatively small drop in volume. This is understandable, since the epithelium already has lost a sizable fraction of its KCl.

When, finally, at the fifth arrow ordinary Ringer replaces nitrate Ringer, the epithelium rapidly recovers the lost volume. As usual the shrinkage below normal value is associated with a drop in potential difference and a reverting to normal potential difference during recovery of the volume.

C. Evidence for Potential- and Volume-Gated Chloride Channels in the Basolateral Membrane

It has been known for a long time that if most or all sodium in the Ringer bathing the inside of the skin is replaced by potassium, the epithelium swells dramatically (MacRobbie and Ussing, 1961; Ussing *et al.*, 1965). The swelling is fully reversible when the high K Ringer is replaced by ordinary Ringer. At first this phenomenon was taken to indicate that the basolateral membrane of the epithelium was permeable to both potassium and chloride ions. It is now clear that the membrane is normally virtually tight to chloride, and that the high chloride permeability is elicited by the depolarization of the membrane. Once the

swelling has started, we get the additional increase in chloride permeability which is always associated with cell swelling. These phenomena are illustrated in Fig. 5. The first steps of the procedure are those used in the foregoing experiments. The outside medium is 1/20 Ringer throughout. At the first arrow, the inside medium is changed from Ringer to half thiocyanate Ringer. The chloride-free epithelium is now exposed to full K Ringer (K^+ replacing Na^+). The chloride-free volume corresponds to 20 μm and the chloride space to 15 μm. Now swelling sets in and the volume is still increasing rapidly when, after 90 min, ordinary Ringer replaces K Ringer. At that moment, the thickness has increased by 80 μm. Immediately after the change of solutions, the epithelial thickness drops precipitously, and in less than 30 min, it is back to the normal value, i.e., that measured at the very beginning of the experiment. Thus, the loss of KCl stops when the cellular chloride concentration still is nearly 50 m*M*. The potential changes during the experiment are consistent with the assumption that both chloride and potassium move through the membrane as ions. It is also characteristic that the swelling reaction seen here is equally large and fast with KNO_3 Ringer, KSCN Ringer, and KI Ringer. In contrast to the cotransport mechanism, the K-associated swelling does not require a specific anion. Under

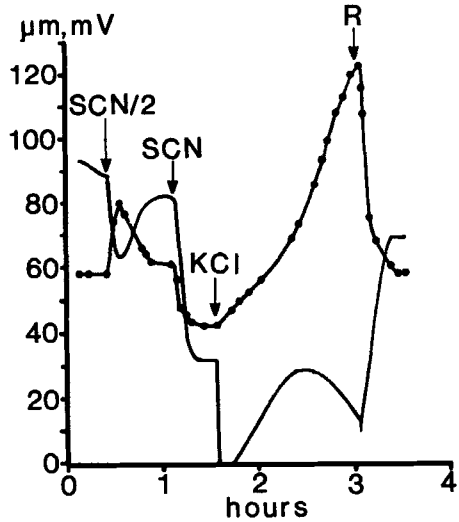

FIG. 5. The basolateral chloride channel is open, even in shrunken cells, when the epithelium is depolarized with high K Ringer. Ordinate and abscissa as in Fig. 2. Outside medium 1/20 Ringer throughout.

At zero time, the inside medium is Ringer. At the first arrow (SCN/2), it is changed to half thiocyanate Ringer. At the second arrow (SCN), it is changed to full thiocyanate Ringer. At the third arrow (KCl), it is changed to potassium Ringer (all sodium replaced by potassium). At the fourth arrow (R), the inside medium is changed back to ordinary Ringer.

suitable conditions, even sulfate is taken up together with potassium (Ussing *et al.*, 1965). We must interpret this as meaning that the anion channels are activated by K depolarization and that they have a low degree of selectivity.

As just mentioned, we may assume that the anion channels are gated by the potential drop following an increased potassium concentration of the medium. As already mentioned, the chloride channels at the apical end of the mitochondria-rich cells are known to be opened by hyperpolarization of the skin, a procedure which depolarizes the apical membrane (see Larsen and Rasmussen, 1982). However, the evidence so far presented here might equally well be explained by an effect of potassium ions on some K^+ receptor on the basolateral membrane of the syncytium cells. One cannot test the depolarization hypothesis directly by clamping the skin potential at a reversed value. Due to the high potassium permeability of the basolateral membrane and the high cellular potassium concentration, the cell interior will remain negative relative to the inside bath, no matter how the potential difference is manipulated. Depolarization of the basolateral membrane can, however, be achieved by closure of the potassium channels, for instance, with Ba^{2+}. In this way, we have obtained evidence for potential gating of the anion channels (see Fig. 6). The initial situation is as in the foregoing experiments. At the first arrow, the Ringer of the bath is replaced by Ringer with 2 m*M* Ba^{2+}. As expected, the potential drops rapidly. At the second arrow, the solution is made half Ringer + 2 m*M* Ba^{2+}. The epithelium swells

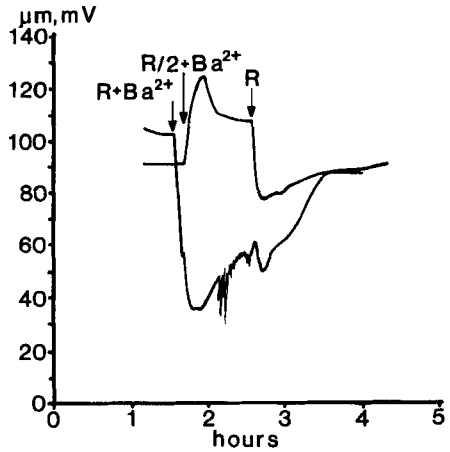

FIG. 6. Closing of the potassium channels with Ba^{2+} during the volume regulation may lead to oscillations of the skin potential, as shown here. Ordinate and abscissa as in Fig. 2. Outside medium 1/20 Ringer throughout. Outside medium originally Ringer.

At the first arrow (R + Ba^{2+}) the bath is changed to Ringer with 2 mmol/liter of Ba^{2+}. At the second arrow R/2 + Ba^{2+}, the bath is changed to half-strength Ringer, made 2 m*M* with respect to Ba^{2+}. At the third arrow (R), the inside solution is again ordinary Ringer.

while the potential difference drops still further. One might have thought that the volume should have remained constant and high. However, as shown by Nielsen (1985), the closure of K channels by Ba^{2+} is followed by a secondary slight increase in K permeability and this, together with the increase in electrochemical potential of cellular K^+ following the strong depolarization, allows K^+ to leave the cells. At the same time, we must assume that the chloride channels are wide open. Thus, there is a loss of KCl, while the potential difference (which is now dominated by the Cl^- diffusion potential) increases *pari passu* with the drop in cellular chloride. After some 30 min, the rate of shrinkage is abruptly reduced and at the very same moment, the potential difference starts oscillating with a frequency of 1–2 excursions per minute. The amplitude often is as large as 20 mV. The trains of oscillations may last for 30 min, or they may be intermittent, but a constant feature (14 experiments) is that strong oscillations start when the potential difference has reached a value between 45 and 55 mV, and that the onset coincides with a drop in the rate of shrinkage of the epithelium. We can formulate the following working hypothesis: Normally the potential across the basolateral membrane is dominated by the potassium diffusion potential. When the chloride channels are open, the potential will drift downward to some value between the potassium potential and the chloride potential. If, however, both chloride channels and potassium channels are closed, we have an unstable situation: The sodium pump will return two potassium ions to the cell for every three sodium ions pumped. With closed potassium channels, the two potassium ions cannot escape. If chloride ions cannot enter, the cell interior must depolarize (or even go positive), until the entry of sodium via the apical sodium channels is brought to a stop. Now, if we assume that the chloride channels are closed when the potential difference is above 50 mV and open gradually at lower potentials, then we can see that the potential is likely to oscillate. Whenever the cellular potential reaches a certain threshold value the channels will close and the pump will drive the potential in the direction of depolarization. It is known that the anion channels of the apical membrane of the mitochondria-rich cells have opening and closure times of the same order as the oscillations seen here, so qualitatively, the hypothesis outlined above makes sense. However, so far the evidence for the assumption that the oscillations are due to the behavior of anion channels is indirect. Clearly, the oscillations ought to vanish if the anion channels were inhibited. We therefore tried the effect on our system of the drug indacrinone, MK196 (see Schlatter *et al.*, 1983), which is known to close the apical chloride channels of the mitochondria-rich cells (Durr and Larsen, 1985). One of our experiments is shown in Fig. 7. At the first arrow, Ringer of the inside bath is replaced by Ringer made 2 m*M* with respect to Ba^{2+} as well as with the anion blocker MK196. The volume remains constant, but the potential difference drops steeply and then partly recovers. The latter process may be related to adjustment of intracellular sodium and potassium concentrations and possibly also to the

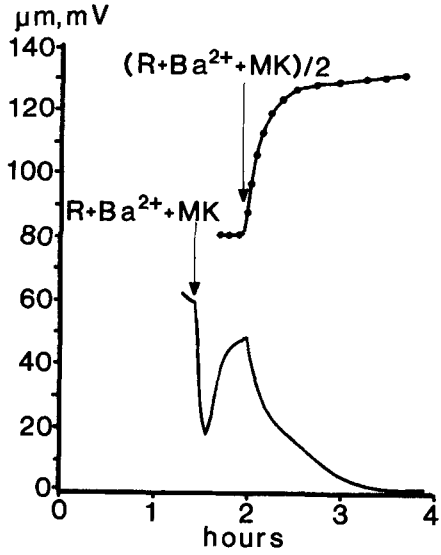

FIG. 7. Chloride channel inhibitor indacrinone (MK196) inhibits volume regulation, as shown. Ordinate and abscissa as in Fig. 2. Outside medium 1/20 Ringer throughout. Inside medium originally Ringer. At the first arrow (R + Ba^{2+} + MK), the inside medium is changed to Ringer made 2 m*M* with respect to Ba^{2+} as well as MK196. At the second arrow (R + Ba^{2+} + MK)/2, the inside medium is diluted to half strength.

opening of a latent potassium pathway following closure of K channels with barium ions (Nielsen, 1985). At the second arrow, the inside medium is diluted to half strength. The volume increases rapidly by about 45 µm and remains high, perhaps showing a very slight increase with time. In fact, this very slight increase was seen in all four of the experiments of this type. The potential difference drops gradually to 0 without showing any oscillations. Thus, the experiment indicates that the oscillations seen when the potential difference drops due to closure of the potassium channels have to do with potential gating of the chloride channels. The fact that the potential difference only gradually drops to 0 is in agreement with the finding that the inhibition of the chloride channels of the apical Cl^- channels of the mitochondria-rich cells also is a slow process. The inhibition seems to be reversible, but the recovery of the potential difference lasts several hours (compare Durr and Larsen, 1985).

III. CONCLUSION

It may be appropriate to consider the handling of potassium separately for the syncytium and the mitochondria-rich cells. Neither cell type performs any net

transport of potassium. To take the syncytium first, both the ion pump and the cotransport tend to accumulate potassium in the cells, but under all physiological conditions the conductance of the basolateral potassium channel is so large that cellular K is not far from electrochemical equilibrium with that of the inside bath.

Thus, the cotransport of $NaKCl_2$, which in other epithelia may lead to net transport of K^+, serves here only as a part of the volume regulation system. This system determines the chloride content of the cells. Since, however, the cation pump keeps the sodium concentration low and reasonably constant, the volume also determines the total potassium content of the cells.

The volume is determined by the relative rates of the cotransport and the latter again is due to the potential-gated anion channels. It is interesting that basolateral, potential-gated anion channels have been found in rabbit urinary bladder by the patch clamp technique (Hanrahan *et al.*, 1984). Just as with the anion conductance described in the present chapter, the one from rabbit urinary bladder has a low specificity so that most small anions can pass.

The mitochondria-rich cells apparently lack the cotransport system. This can be concluded from the fact that the transepithelial chloride transport is strictly passive, following the flux ratio equation (compare Willumsen and Larsen, 1985). The cotransport in combination with the cation pump would result in a secondary active outward transport of chloride ions which, incidentally, would be disastrous for the animal.

ACKNOWLEDGMENT

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Chapter 2

Na^+, K^+, and Rb^+ Movements in a Single Turnover of the Na/K Pump

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I. INTRODUCTION

The active transport of K^+ and Na^+ in the kidney as well as in all other mammalian tissues is driven by the chemical energy of ATP and is carried out by the Na/K pump, an enzyme in the cell membrane also known as Na^+,K^+-ATPase. Since the discovery by Skou of the Na^+,K^+-ATPase in 1957, intensive efforts in many laboratories have been directed toward gaining an understanding of the molecular mechanism by which the active transport process is carried out. Today we have an excellent phenomenological description of the ion movements, of the hydrolysis of ATP, and of a number of enzymatic events in the transport cycle (cf. Glynn, 1985); we also have a general understanding of how conformational changes in a protein molecule could be orchestrated to bring about the vectorial movements of Na^+ and K^+, linked to the process of ATP hydrolysis. The hope is that in the next few years it will possible to work out the details of the kinetic mechanism, including events involved in ion binding and release, and to assign the affinities and conformational changes to physical sites and configurations in the now emerging structure of the Na^+,K^+-ATPase (Shull *et al.*, 1985; Kawakami *et al.*, 1985; Herbert *et al.*, 1985).

Given the complexity of the Na/K pump molecule, it should not be surprising that many significant questions regarding the pump mechanism remain unanswered. For instance, at least 10 ligands of the Na^+,K^+-ATPase can be enumerated: $3Na^+$, $2K^+$, ATP, ADP, P_i, Mg^{2+}, and ouabain. While, on the one hand, similar ligands may occupy the same binding site at different times, on

[1] With the technical assistance of Grace Jones and John T. Barberia.

the other hand, there is evidence for additional "modifying sites" for some of the ligands. Furthermore, the binding sites undergo marked changes in affinity and accessibility at various points in the catalytic cycle, which is reflected in apparently different "inside" and "outside" sites for the cations. Thus, detailed kinetic models must be very large: A conservative model of the K^+-transporting *half*-cycle of the pump includes 20 states (Karlish *et al.*, 1982), without including the interactions of Mg^{2+}, without distinguishing the two K^+ binding sites, and without considering simultaneous binding of Na^+ at modifying (or transport?) sites.

We will use the very much simplified kinetic model (Albers *et al.*, 1968; Post *et al.*, 1969) shown in Fig. 1 as a framework for discussion of the current view of

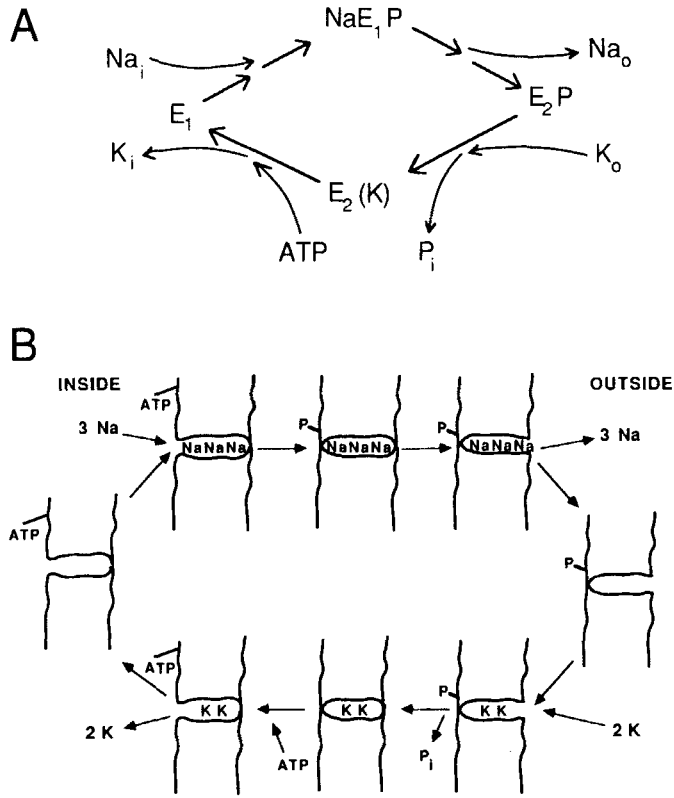

FIG. 1. (A) Simplified biochemical model of Na^+,K^+-ATPase. (B) A diagram corresponding to A and emphasizing the ion movements in a reaction cycle of the Na/K pump. Note that a ping–pong model is illustrated in which only one set of binding sites alternates between the transport of Na^+ and K^+.

the Na/K pump and to point out areas of uncertainty. The reader is referred to the excellent recent review by Glynn (1985) for a more extensive analysis and for appropriate references. Note that the cartoons in the lower panel parallel the more conventional diagram above and are used to emphasize the interactions of Na^+ and K^+ with the pump molecule. Starting on the upper left, Na ions are bound to the intracellular face of the pump along with MgATP and a transfer of phosphate from ATP to an aspartyl residue on Na^+,K^+-ATPase takes place. This is followed by a conformational change resulting in exposure of the Na sites to the extracellular medium and to Na^+ release. Reversal of this half of the cycle in the presence of extracellular Na^+ (and no K^+) and intracellular ADP is thought to account for the Na_i–Na_o exchange reaction observed in steady-state flux measurements. K^+ binds at extracellular sites to the phosphorylated Na^+,K^+-ATPase and brings about rapid dephosphorylation; then, following binding of ATP to a low-affinity site, a conformational change takes place exposing the K sites to the intracellular medium, and K^+ dissociation completes the cycle. As was the case with the Na half-cycle, reversal of the K half-cycle is seen in flux measurements as a K_i–K_o exchange flux in the presence of intracellular ATP and P_i.

Uncertainties remain at many levels. While the Albers–Post scheme in Fig. 1 is the most widely accepted framework, even its most fundamental aspects are still open for discussion. (1) There has been considerable concern in recent years that under physiological conditions, the proposed phosphorylated intermediates are not "kinetically competent" and are merely a side reaction; recently, attention has been focused on the proposition that E_1-P cannot be involved in the cycle (cf. Norby, 1985). This will not be considered further here. (2) Because of the complexity of the system (including alternate pathways of E-P breakdown), it has not been possible to distinguish by means of steady-state kinetic analysis between models in which Na^+ and K^+ ions are transported one after the other, as in Fig. 1, from models in which both sets of ions are transported at the same time. Experiments regarding the transient behavior of the Na^+,K^+-ATPase are generally used to support the ping–pong-type mechanism, but Skou (1985) has recently argued that the data can be interpreted in favor of a model involving simultaneous transport of the two ions. Measurements reported here will be seen to favor the transport of Na^+ at an earlier step than the transport of K^+. (3) Taken literally, Fig. 1 suggests that Na and K transport sites are never occupied at the same time. This is an important issue because it is appealing to think that there might be only one set of binding sites with specificity for Na^+ or for K^+, depending upon enzyme conformation, as would be inferred from a literal acceptance of the cartoons in Fig. 1. However, available data do not preclude simultaneous occupancy of either internal or external sites for the different transported ions (cf. Sachs, 1985; Skou, 1985). Experiments presented here address this question, but the answer is not yet conclusive.

Our current understanding of the events of ion binding, translocation, and dissociation within the catalytic cycle of Na^+,K^+-ATPase is limited by the fact that until recently only steady-state measurements of ion movements have been possible. Thus, the location of the ion transport events within the reaction scheme has been inferred largely from steady-state kinetic analysis and by studying the role of the ions in affecting the transient kinetics of Na^+,K^+-ATPase phosphorylation and conformational change. A much better understanding of the Na/K pump mechanism will be possible if the individual events of ion transport can be resolved on the time scale of a single reaction cycle, which occurs in about 50 msec at 20°C (cf. Forbush, 1984b). Recently, we have found that such measurements are possible using a preparation of rightside-out membrane vesicles from dog kidney with a very high Na/K pump density (Forbush, 1982) and utilizing a novel rapid filtration apparatus for determination of the rate of isotopic vesicles (Forbush, 1984a). Our recent attempts to resolve Na and K (or Rb) transport in the first turnover of the pump are summarized below.

An important feature of recent models of the Na/K pump is the existence of occluded states in which Na^+ or K^+ ions, in the process of being translocated, are tightly bound and are accessible from neither side of the membrane; this is schematized in the center cartoons in the lower panel of Fig. 1. Post *et al.* (1972) first demonstrated the existence of such a state in the K limb of the cycle by showing that the Na^+,K^+-ATPase could "remember" a prior exposure to K^+ as compared with other cations; the inhibition of phosphorylation brought about by exposure to K^+ could be relieved by exposure to ATP at a low-affinity site. Glynn and Richards (1982) have shown directly that ^{86}Rb ions can be tightly bound to the Na^+,K^+-ATPase and that the characteristics of binding are consistent with the transport model. Evidence will be summarized below that the rate of release of ^{86}Rb is consistent with the occluded state as an intermediate in the Na/K pump cycle and that it is even possible to distinguish two distinct K sites by their rates of release of ^{86}Rb under certain conditions.

Throughout most of the work described in this chapter, Rb has been used in place of K because of the high cost and inconvenience of using high specific activity ^{42}K. All of our principal observations made with ^{86}Rb have been confirmed with ^{42}K, although in much less detail. We find, as have others, that the ions are qualitatively identical, although Rb is released more slowly than K from the occluded state (see Post *et al.*, 1972, and below).

II. METHODS

The use of a rapid filtration apparatus to measure isotopic efflux from membrane vesicles and the release of ligands from membrane proteins has been previously described (Forbush, 1984a), and details of its application to studies of

Na^+K^+-ATPase have been presented (Forbush, 1984b, 1985, 1986a,b). This method offers very great advantages in terms of signal-to-noise ratio and in conservation of membranes as compared to conventional rapid reaction methods. As outlined in Fig. 2, the procedure is simple to use. Briefly, vesicles are loaded with isotope or broken membranes are incubated with ligands under conditions leading to isotopic uptake or binding. A small sample, typically 4 μl containing 20 μg protein, is diluted in 200 μl of appropriate medium, filtered onto a spot 4 mm in diameter on a Millipore filter (HAWP, or Gelman GN-6), and rinsed to remove isotope not associated with the membranes (typically 99%). The filter and membranes, still wet, are transferred to the rapid filtration apparatus where filtration is continued under pressure. The filtrate, which squirts from the filter funnel in a fine stream, is collected in a set of 56 cuvettes spinning on a phonograph turntable; it constitutes a nearly continuous record of the rate of isotopic efflux from vesicles or release of radioligand from membranes. Usually, filtrate is collected for only one revolution of the turntable during which a change is made in the sample: Either a flash of light is used to release caged ATP (Kaplan *et al.*, 1978) within the vesicles or the solution flowing past the sample is changed rapidly from "solution a" to "solution b." With present versions of

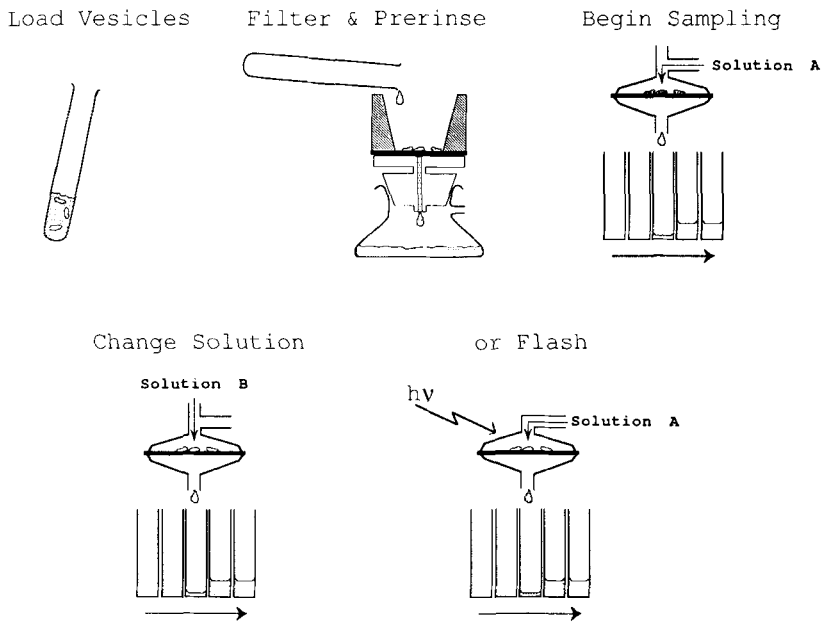

FIG. 2. A schematic diagram illustrating the rapid filtration method for measurement of isotopic efflux from membrane vesicles or of dissociation of bound ligands from membrane proteins. For explanation, see text.

the apparatus, the time resolution is 10–20 msec, including the time required for changeover of solution or delivery of a flash.

The preparations of tight rightside-out membrane vesicles from dog kidney outer medulla (Forbush, 1982), of inside-out membrane vesicles from eel electroplax (Forbush, 1985), and of detergent-washed membranes rich in Na^+,K^+-ATPase ("broken membranes," Forbush, 1985) were performed as previously described. In the preparation of kidney vesicles, a minor modification of the homogenization procedure has led to a considerable increase in the fraction of membranes that are isolated as tight vesicles: Instead of the regular Teflon pestle homogenizer, a machined-down Teflon pestle has been used which tapers from a 2-mm clearance (with serrations) at the bottom of the homogenizer to a 0.2-mm clearance at the widest point in the pestle. We initially tried this pestle to ease the homogenization of the dissected kidney pieces, and for this it is quite effective. We have also found that by using it for 5 strokes at ~1200 rpm, most of the membranes destined to vesiculate are released from the tissue—more violent homogenization using the regular homogenizer doubles the yield of Na^+,K^+-ATPase, but at little increase in the yield of vesicles.

III. Na FLUX IN A SINGLE TURNOVER OF THE Na/K PUMP

To examine the time course of ^{22}Na efflux within a turnover of the Na/K pump, we have utilized a preparation of rightside-out vesicles from dog kidney outer medulla (Forbush 1982). Since Na^+,K^+-ATPase constitutes upward of 50% of the membrane protein in some of these vesicles, a single turnover of all of the pumps should theoretically result in a loss of ~0.3–1.0 m*M* Na from within the vesicles. In order to synchronize the pumps, the vesicles are preloaded with caged ATP and ^{22}Na, conditions under which the Na^+,K^+-ATPase should be in the Na-E_1 state; then to start the hydrolysis cycle, ATP is released in a bright flash of light. ^{22}Na efflux is monitored continuously by collecting medium that flows past the vesicles at a high rate; utilizing the rapid filtration apparatus described previously (Forbush, 1984a), the time resolution is ~10–20 msec. Two types of experiments have been performed using two different light sources. In our first experiments, a brief exposure (14–33 msec) to a continuous arc lamp was used to release less than ~50 μ*M* ATP from a much larger pool of caged ATP; the exposure could be repeated on successive rotations of the rapid fraction collector, and the efflux data were effectively "signal averaged." In each flash, fewer ATP molecules were made available than the number of Na^+,K^+-ATPase molecules, so that only a single cycle of pumping could occur following each flash. More recently, we have used a bright flash lamp to release >80% of the ATP in a single exposure of less than 1 msec. In this case, the amount of

available ATP is much larger than the number of pumps so that multiple turnovers of the pump can take place, and we should observe a steady state following initial transients.

The result of an experimental run utilizing the repetitive weak flash protocol is shown in Fig. 3A. ^{22}Na efflux averaged over 6 cycles of the turntable is plotted as the number of counts in each of the sampling cuvettes. In each cycle of the turntable, a 33-msec flash was presented at time equals 0 sec, and immediately following the flash a rapid increase in the rate of ^{22}Na efflux is seen, followed by a slower exponential decay. The burst is dependent upon the action of the Na/K pump, since it is completely absent if the vesicles have been preincubated with ouabain (Fig. 3B). Other experiments confirmed the expectation that the burst represented Na movement in a single turnover when it was found that the time course of the burst was independent of the amount of ATP released in a flash, over the range estimated to be 25–200 μM release in the vesicles. Further support is provided by experiments utilizing the single bright flash protocol, discussed below.

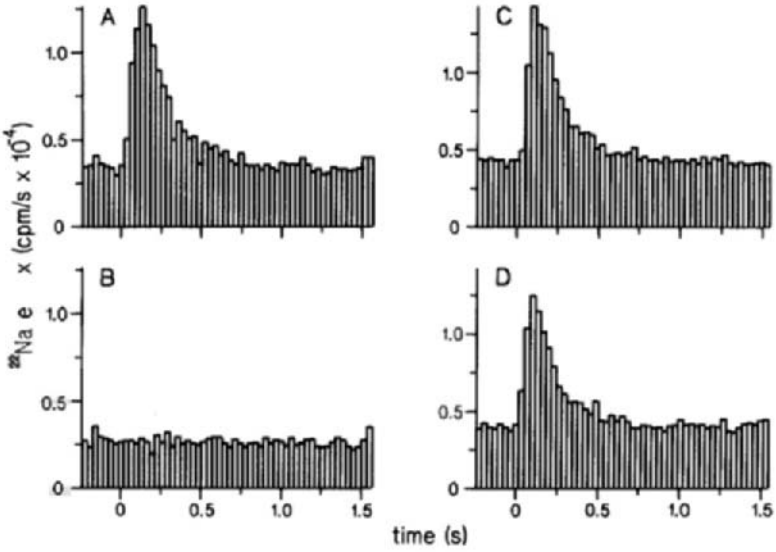

FIG. 3. ^{22}Na efflux from renal membrane vesicles following a flash of light to release ATP from caged ATP. (A and B) are from an experiment at 15°C in which six cycles were averaged; a 33-msec flash was given in each cycle, at time = 0 sec (no compensation for system delay). (B) The sample was preincubated in 2 mM ouabain. (C and D) are from a different experiment in which eight cycles were averaged. In C, the efflux medium contained 125 mM sucrose/33 mM Tris-HEPES, pH 7.5; in D, it contained in addition 10 mM K^+.

External K^+ was found to have no effect on the time course of the ^{22}Na efflux burst, as illustrated by a comparison of Fig. 3C and D. This is consistent with external K^+ binding to the Na/K pump *after* Na is released from extracellular sites, as in the usual versions of the Albers–Post model. But the result says only that external K^+ does not affect ^{22}Na release, which would also be the case if both ions could bind at the same time and if the binding of K^+ did not alter the slow steps in Na translocation.

By examining the time course of the ^{22}Na efflux burst at different temperatures, it was found that the rise of the efflux rate to a maximum was slower than could be accounted for by the mixing time of the rapid filtration apparatus. Thus, the burst of ^{22}Na efflux could be well fit by a sequence of (at least) two first-order processes, the first leading to formation of the state which releases Na from the extracellular face of the membrane, and the second leading to the breakdown of that state. We estimate from experiments similar to that illustrated in Fig. 3 that at 10°C, rate constants of the rise and fall of the efflux are ~ 30 sec^{-1} and ~ 4 sec^{-1} (at 20°C, >100 sec^{-1} and ~ 40 sec^{-1}). It seems reasonable to assign the rate constants to the two steps in the model, but it must be pointed out that the prediction of the model is the same when the order of assignment is reversed.

If all of the caged ATP were released rapidly, there would be an excess of ATP molecules over Na/K pumps, and multiple pump cycles should occur. Using the bright flash lamp, it was possible to photolyze almost all of the caged ATP in a single flash. In Fig. 4, which shows the result of such an experiment, it is seen that the burst of ^{22}Na efflux is almost identical to the burst observed with the weak flash lamp; now, however, a maintained rate of Na^+ efflux was also observed following the burst; even this rate declines with time as the available ATP and $^{22}Na_i$ are exhausted. The occurrence of a "pre-steady-state" burst is strong evidence that Na transport is an early event in the overall pump cycle. In similar experiments (not shown), we found that the time constant of the burst was unaffected by extracellular K^+, as we had found with the weak flash protocol, but that the steady-state rate was increased by K^+, consistent with the known requirement for K^+ in the full turnover.

The slow release of ^{22}Na from the outside face of the Na/K pump is surprising in view of the high rate of the biochemical steps presumed to accompany the Na limb of the transport cycle. It is generally assumed that Na is released from an outward-facing phosphorylated form of the pump, usually considered to be E_2-P. The formation of the phosphorylated intermediate from ^{32}P-ATP and the conformational change from E_1-P to E_2-P have been shown to proceed with an overall rate constant greater than 100 sec^{-1} in Na^+,K^+-ATPase from eel electroplax (Hobbs *et al.*, 1985). More importantly, the K-stimulated dephosphorylation of E_2-P is also rapid, so that at 20°C the entire phosphorylation–dephosphorylation sequence should have a rate constant >100 sec^{-1} in eel Na^+,K^+-ATPase (Froelich *et al.*,

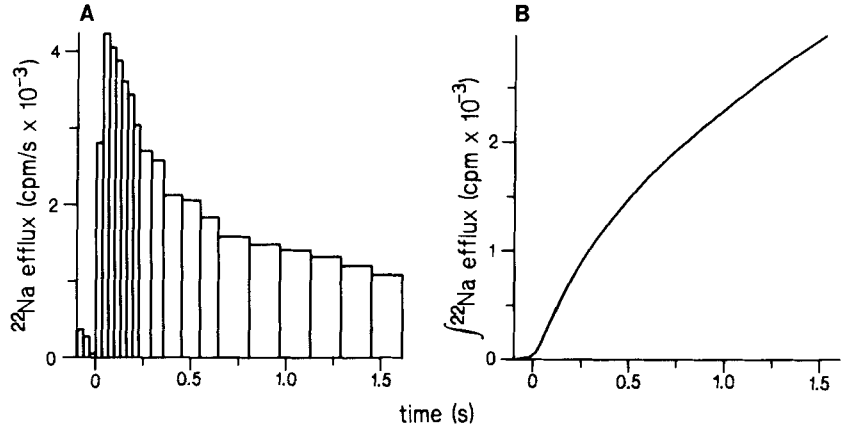

FIG. 4. ^{22}Na efflux following a bright flash of light to release almost all of the ATP from caged ATP. The flash was given at time = 0 sec, and effluent was collected for only a single cycle of the turntable. (B) Time integral of the rate of efflux shown in A. Temperature 15°C.

1976) and >60 sec^{-1} in beef brain Na^+,K^+-ATPase (Mardh, 1975). Our observed time course of the Na efflux burst is slower than this about 2-fold. Two possible explanations for the discrepancy can be suggested: (1) There may be a substantial species difference in Na transport steps between the dog kidney Na^+,K^+-ATPase on which these studies were performed and the eel electroplax and beef brain enzymes for which the phosphorylation data are available. It will be of great importance in future studies to obtain parallel records of Na translocation and P_i release from E_2-P with the same preparation of Na^+,K^+-ATPase. (2) Alternatively, the data can be used to support the hypothesis that Na is released from the Na/K pump after K has bound and phosphorylation has proceeded. For this to be so, the transport sites for Na and K would have to be physically distinct, a conclusion which would be of fundamental importance in pump mechanism, but which would certainly be premature in view of the species difference. Again, while at first sight it might appear that the lack of effect of K on the rate of the Na burst is convincing evidence *against* simultaneous occupancy of distinct Na and K sites, the finding is also consistent with K binding before Na is released, but only if occupancy of the outside K sites does not affect the slow steps in Na transport.

Two other lines of evidence are in support of a slow release of Na from the extracellular face of the Na/K pump. The first comes from recent studies of Karlish and Kaplan (1985) in which the influx of ^{22}Na through inside-out pumps in reconstituted vesicle systems was determined at 0°C: A pre-steady-state burst of Na movement was seen with a decay of ~0.05 sec^{-1}, much slower than E-^{32}P formation determined in parallel experiments. The authors ascribe this to a slow transition between $Na \cdot E_1$-P and $Na \cdot E_2$-P. Although the rate constant for the

breakdown of E-P was not reported, we have found that for SDS-treated Na^+,K^+-ATPase from dog kidney and 0.2 m*M* [γ-^{32}P]ATP, the rate of decay from the E-^{32}P level in the presence of Na and K is ~0.08 sec^{-1} at 0°C (unpublished results). Considering the scatter in the time constants in both types of measurements, the difference is probably not significant. Second, recent studies of Beeler and Keffer (1984) have shown that Ca^{2+} release from the intravesicular face of the sarcoplasmic reticulum Ca-ATPase is much slower than the events leading to formation of $Ca{\cdot}E_2$-P. In view of the strong homology between the structure and kinetics of Ca-ATPase and of Na^+,K^+-ATPase, this supports by analogy the finding of slow Na^+ release.

There is one caveat which must be presented with regard to our finding of a slow time course of Na efflux. Since caged ATP binds to the high-affinity site on Na^+,K^+-ATPase with about 40-fold lower affinity than does ATP, it must occupy the active site prior to photolysis (Forbush, 1984b). With the bright flash lamp, enough light is given to photolyze all of the caged ATP, and we presume that the ATP at the active site is photolyzed. But the photolysis of caged ATP occurs as an acid-catalyzed dark reaction following photoactivation; the dark reaction has a rate of ~150 sec^{-1} at pH 7.2 (McCray *et al.*, 1980), not slow enough to present a problem in the present experiments. Thus, it is possible that the hydrolytic cleavage of the compound proceeds more slowly when the molecule is bound to the active site. If so, in the limiting case the caged ATP would not be hydrolyzed at all while it occupied the site, but it would dissociate from the site and be replace by free ATP that had been produced in bulk solution. As discussed previously (Forbush, 1984b), it seems likely that this latter sequence of events would proceed much more rapidly than 50 sec^{-1}, but it will be necessary to confirm this in future experiments in which we monitor the delay in enzyme phosphorylation following the addition of ^{32}P to caged ATP and Na^+,K^+-ATPase.

IV. ^{86}Rb FLUX IN A SINGLE TURNOVER

In order to study transport of K^+ and its release from the intracellular surface of the membrane, it is necessary to utilize a preparation of inside-out membrane vesicles. So far, we have been unable to obtain a significant fraction of inside-out vesicles from renal outer medulla despite many alterations in homogenization technique and solutions. Instead, we have characterized a vesicle preparation obtained by sonication and Hypaque centrifugation (Forbush, 1982) of a plasma membrane preparation from eel electroplax. The Na^+,K^+-ATPase content of this preparation is 30–50% of membrane protein, at least as high as in the mammalian outer medulla preparation. The vesicles are very leaky to Na, as no trapped Na space is detected by filtration after a 2-hr loading period, but the

vesicles are reasonably tight to ^{42}K and ^{86}Rb, releasing the latter with a 2-min half time at 20°C. Exposure of ^{86}Rb-loaded vesicles to Mg^{2+} and ATP leads to a loss of 60–70% of the isotope within 5 sec, consistent with intracellularly directed ^{86}Rb flux in inside-out vesicles. The ATP requiring flux is inhibited by vanadate, but not by ouabain, further consistent with a pump orientation in which only the intracellular face is accessible to the medium.

Using the eel electroplax plasma membrane vesicles, it has been possible to study rapid ^{86}Rb efflux in the rapid filtration apparatus. Vesicles were preloaded with ^{86}Rb, rapidly washed, and transferred to the apparatus. Instead of delivering ATP from caged ATP in a flash of light, MgATP could be introduced rapidly into the filtration chamber by switching the filtration medium to a medium containing MgATP. Unfortunately, this switchover is accompanied by a significant step increase in hydrostatic pressure, which is sufficient to dislodge or break some of the vesicles and create a short pulse of ^{86}Rb in the efflux record. This "pressure artifact" is shown in the upper panel of Fig. 5. Although it is not constant in magnitude from sample to sample, it is easily distinguished from changes due to composition of the solution, since it begins when the valve is actuated, some 30 msec before the new solution arrives at the filter. Note also that in ^{86}Rb efflux experiments, it is important to anticipate a contribution from release of ^{86}Rb that is tightly bound to Na^+,K^+-ATPase, rather than in the vesicle interior, prior to the time course. Since, as discussed below, such "occluded" ions are released on addition of ATP without Mg^{2+}, the prerinse solution in these experiments included 4 m*M* ATP. This ensured that all of the Na/K pumps were in the $E_1 \cdot$ATP state (not shown explicitly in Fig. 1) prior to the change of solution. Further progress in the cycle was blocked, since phosphorylation of the Na^+,K^+-ATPase absolutely requires Mg^{2+}.

When Mg^{2+}, in addition to Na^+ and ATP, were added to the outside of inside-out membrane vesicles preloaded with ^{86}Rb, an increase in ^{86}Rb efflux was seen, as shown in Fig. 5B. After subtraction of the pressure artifact, the resulting record of MgATP-dependent ^{86}Rb efflux shown in Fig. 5C is obtained: After the solution is switched at time equals 0, there was a rise to a more or less steady state of ^{86}Rb efflux. It is clear that there is no pre-steady-state burst of efflux comparable to the above case with Na transport, although the efflux of ^{86}Rb decreases with time as the internal pool of ^{86}Rb is exhausted. This indicates that the Rb (K) translocation steps include (or are later than) the major rate-limiting steps in the cycle, consistent with previous suggestions that the breakdown of the E(K) occluded transport intermediate is rate limiting (Karlish and Yates, 1978).

Close examination of the time course in Fig. 5C reveals that ^{86}Rb efflux from the inside-out vesicles does not begin immediately upon addition of Mg^{2+} to initiate ATP utilization, but proceeds after a short lag. We have found this to be a consistent feature in each of 11 experiments, although as in the case shown here,

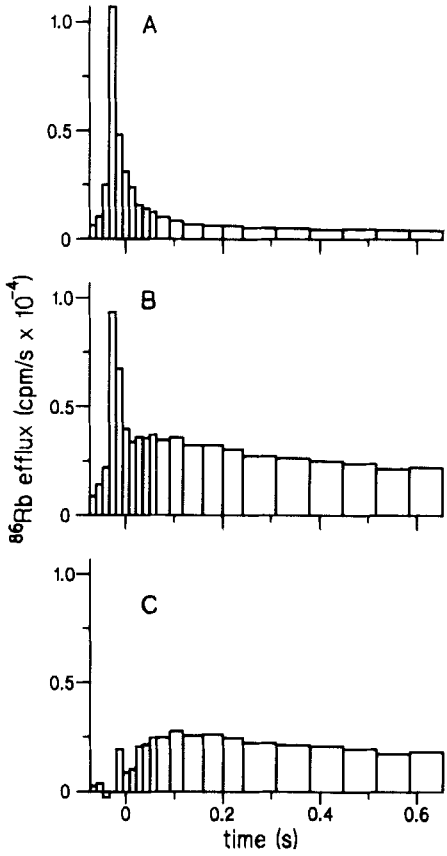

FIG. 5. ^{86}Rb efflux from inside-out membrane vesicles from eel electroplax. Solution a contained 60 m*M* Na/5 m*M* ATP/250 m*M* sucrose/25 m*M* imidazole/6 m*M* EDTA. The results of eight independent runs at 10°C are averaged. (A) Solution b contained in addition 5 m*M* Mg^{2+} and solution b was the same as solution a. (C) Difference between A and B. Time = 0 is marked as the time the solution change effectively reaches the filter as determined from counts of ^3H_2O in the second solution (not shown, see Forbush, 1984a).

the data are "noisy" at the early time points due in part to the pressure artifact. From these experiments, we estimate the rate constant of the increase in ^{86}Rb efflux to be ~20 sec^{-1} at 10°C. This is consistent with the delay that would be predicted from the series of steps involving phosphorylation of the enzyme followed by E_1–E_2-P transition and K^+ binding, which, as noted above, are thought to be very rapid in eel Na^+,K^+-ATPase (>50 sec^{-1} at 20°C). On the other hand, when compared to the time course of ^{22}Na efflux from kidney vesicles at a comparable temperature (not shown), it is apparent that the ^{86}Rb

movement reaches a maximum much more rapidly than did the decay of the ^{22}Na burst in the membrane vesicles from dog kidney. This reiterates the point made above that either there are substantial species differences in the rate of the Na transport steps or else the Na and Rb transport steps overlap, implying simultaneous occupancy of outside Na and K sites.

In summary, the ^{22}Na and ^{86}Rb rapid flux experiments support models of the Na/K pump in which the transmembrane transport of Na is an early step in the pump cycle initiated by ATP and in which steps involving K movement come later and involve the overall rate-limiting step. However, if we accept the assumption that caged ATP is "well behaved" at the active site, the data also indicate that the release of Na from sites on the extracellular face of the pump is slower than expected, either because of a species difference or because Na is not released before the K sites become occuped. If the latter is the case, it appears that there is little interaction between the two sets of extracellular sites, since the slow step in Na movement is not significantly affected by K binding.

V. RELEASE OF K^+ OR Rb^+ FROM A TRANSPORT INTERMEDIATE

In order to assess the role of the occluded state in the normal Na/K pump cycle, we have studied the breakdown of E_2 (^{42}K) and E_2 (^{86}Rb) in SDS-washed kidney microsomes using the rapid filtration apparatus (Forbush, 1984a, 1986a,b). Broken membranes were preincubated with ^{42}K or ^{86}Rb to form the occluded state of Na^+,K^+-ATPase, and after briefly washing away excess isotope, the membranes were transferred to the rapid filtration apparatus and washed with solutions containing various ligands. The rate of ^{42}K release as a function of time after initiation of flow of solution b is plotted on a logarithmic scale in Fig. 6. It is seen that ^{42}K was rapidly released from the enzyme upon washing with Na and ATP (Fig. 6A), but only very slowly in the absence of ATP (Fig. 6B). The action of ATP was found to be at a site with ~0.4 m*M* affinity (Forbush, 1986a), in agreement with the value estimated by Karlish and Yates (1978) for the effect of ATP on the $E_2(K)$ form identified by intrinsic fluorescence of Na^+,K^+-ATPase. Although MgATP was found to be twice as effective as saturating concentrations of ATP alone, Mg^{2+} was not absolutely required, and both ADP and MgADP were effective as well, demonstrating, in accord with previous results, that phosphorylation is not involved in breakdown of the occluded state promoted by nucleotide.

The maximal rate constant of ^{42}K deocclusion (fit by nonlinear regression to a single exponential process) was 35–55 sec^{-1} in different preparations of Na^+,K^+-ATPase, while for ^{86}Rb (not shown), it was 13–30 sec^{-1}. Since the pump turnover rate in optimal Na^+ and K^+ at 20°C is ~20 sec^{-1} (cf. Forbush,

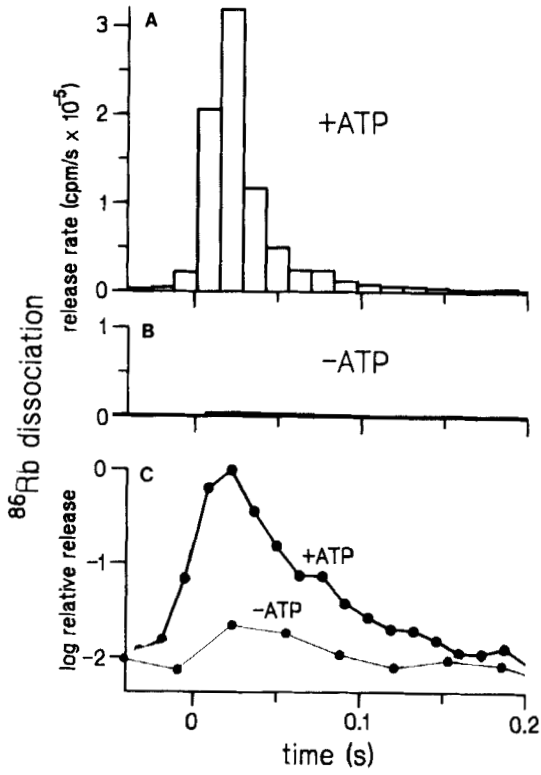

FIG. 6. ^{86}Rb release from an occluded state of the Na/K pump. Broken membranes containing ^{86}Rb tightly bound to Na^+,K^+-ATPase were analyzed in the rapid filtration apparatus (for details, see Forbush, 1984a). Solution a contained 75 m*M* Na, 25 m*M* K, and 25 m*M* imidazole/1 m*M* EGTA, pH 7.2. Solution b contained in addition either 4 m*M* Mg and 4 m*M* ATP (A) or 4 m*M* Mg (B). (C) Logarithm of the rate of ^{86}Rb release, from the data in A and B.

1984b), it is thus clear that the release of occluded ions is fast enough for the occluded state to be an intermediate in the normal pump cycle, but that even in the presence of ATP, the release process must be a significant rate-limiting step. Karlish and Yates (1978) have previously reached the same conclusion regarding the kinetic competency of the $E_2(K)$ form monitored by fluorescence. The point is made even more strongly in experiments in which we have examined the pH dependence of ^{86}Rb deocclusion and Na^+,(K/Rb)-ATPase activity. The pH profile for the Na^+,Mg-ATP-stimulated release of ^{86}Rb is shown in the top of Fig. 7; it illustrates a monotonic increase in the rate of ^{86}Rb release between pH 6.0 and 8.6. Below pH 7.5, the overall rate of the ATPase reaction also increases with pH and is strongly affected by whether K^+ or Rb^+ is present in the

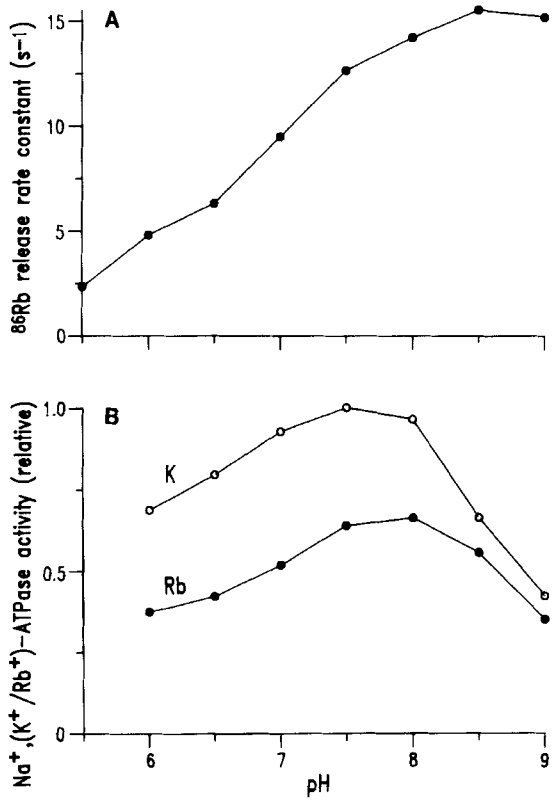

FIG. 7. The pH dependence of the rate of ^{86}Rb deocclusion and Na^+,K^+-ATPase activity. ^{86}Rb release was measured determined in the presence of NaMgATP (A), as in Fig. 6. Na^+,K^+-ATPase activity (B) in the presence of 120 m*M* Na^+ and 25 m*M* of either K^+ or Rb^+ at 20°C. Since the site density of Na^+,K^+-ATPase was not determined, the Na^+,K^+-ATPase activity is expressed in relative units.

medium: This behavior is consistent with the breakdown of $E_2(K)$ or $E_2(Rb)$ as the rate-limiting step between pH 6.0 and 7.5. At higher pH, however, the overall ATPase reaction declines with increasing pH and must be limited by some step other than deocclusion; significantly, the difference between the rate in the presence of K and Rb almost disappears by pH 9.

The study of the occluded state presents an excellent opportunity to study multiple ion binding sites on Na^+,K^+-ATPase, because in this state the K^+ transport sites are fully occupied: Any action of other cations on the initial rate of deocclusion must be exerted at sites other than these. Thus, in other experiments we have found that in the presence of ATP or ADP, the cations Na, K, and Rb

(but not Li) each stimulate (~2×) the rate of deocclusion in replacement for choline or *N*-methylglucamine. This demonstrates the coexistence with the occlusion sites of moderate affinity sites (4–15 m*M*) for other cations. At this point, we have no idea if these sites are on the cytoplasmic or extracellular face of the Na/K pump, or, of course, if these sites are transport sites. It may be noted that even in the simplest ping–pong models of transport in which sites alternate to bind 3 Na^+ or 2 K^+, one of three sites may be available for ion binding, but may not be involved in transport during the K^+ limb of the cycle.

As indicated in the model of Fig. 1, the K^+ transport steps of the reaction cycle are thought to be reversible in the presence of Mg^{2+} and P_i to phosphorylate the enzyme; for instance, the Rb–Rb exchange reaction in red cells (Glynn *et al.*, 1970) and reconstituted kidney Na^+,K^+-ATPase (Karlish *et al.*, 1982) require $Mg^{2+}P_i$ for maximal activity, presumably to promote the release of Rb to the extracellular face of the membrane. As illustrated in Fig. 8, we have found, as have Glynn and Richards (1983), that exposure of the occluded state to Mg^{2+} and P_i leads to rapid release of ^{86}Rb.

A surprising result was obtained when K^+ rather than Na^+ was present in the

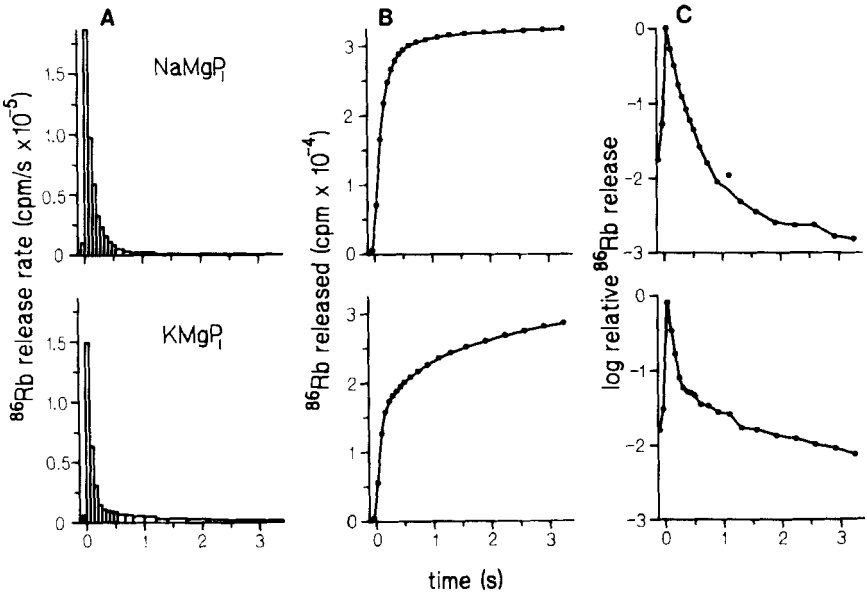

FIG. 8. The release of ^{86}Rb from the occluded state in the presence of Mg, P_i, and either Na^+ (upper panels) or K^+ (lower panels). (A) Rate of ^{86}Rb release as a function of time. (B) Time integral of the same data showing the amount of ^{86}Rb that has dissociated as a function of time. (C) Logarithm of the rate of ^{86}Rb release, from the same data.

medium: The dissociation was found to be very markedly biphasic, with the two rate constants separated by about an order of magnitude. The plot of amount of dissociation versus time (Fig. 8, bottom center) clearly indicates that about half of the ^{86}Rb is involved in each phase. This result explained the discrepancy between our observation that all of the occluded ^{86}Rb was released with $Mg^{2+}P_i$ and the report of Glynn and Richards (1983) that only 50% was released; the same explanation has been reached independently by Glynn and co-workers (1985). The rate of the slower phase of ^{86}Rb dissociation is lowered by K^+ at a site with high apparent affinity ($K_{1/2} \cong 50\ \mu M$, not shown), suggesting that this action of K^+ is at a K^+ transport site on the extracellular face of the Na/K pump. Since this must be an occulusion site, it seems likely that there are two nonidentical sites for Rb occlusion (sites "s" and "f"), the release of ^{86}Rb from the s site only being rapid if the f site is unoccupied. This model, shown in Fig. 9, predicts that the K^+ (or Rb^+), which acts to stabilize half of the ^{86}Rb, must itself act at the occlusion site and potentially become occluded.

We have used a sequence of incubations with unlabeled and labeled Rb to test the ideas presented in Fig. 9. First, we bound ^{86}Rb to both sites in an incubation with ^{86}Rb and MgP_i and then allowed the f site to exchange with unlabeled Rb for 5 sec at 5°C, leaving only the s site labeled. The sample was then diluted in buffer, filtered, and washed to remove all but tightly bound Rb. When release of

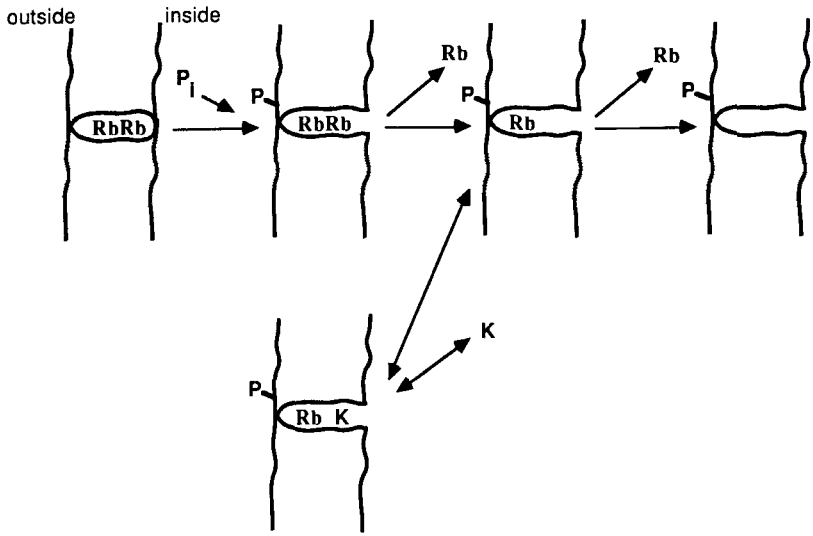

FIG. 9. Diagram illustrating the idea of two nonidentical K^+ sites, the release of ^{86}Rb from the s site being blocked by occupancy of the f site.

^{86}Rb into $KMgP_i$ was measured in the rapid filtration apparatus, the result shown in Fig. 10B was that only the slow phase of ^{86}Rb release ascribed to the s site was observed. On the other hand, when the position of the radioactive label was reversed in the incubation sequence, the fast phase of the f site is seen (Fig. 10C), illustrating that the ^{86}Rb in the second incubation became occluded. Finally, it may be noted that the algebraic sum of these two phases (Fig. 10D) adequately accounts for the time course of release in the control sample (Fig. 10A).

In the presence of $KMgP_i$, the release of ^{86}Rb is fast enough (8–18 sec^{-1}, depending on the preparation) for the occluded state to be an intermediate step in the ATP + P_i-stimulated Rb–Rb exchange (~7 sec^{-1}; Karlish *et al.*, 1982). It is interesting to note that following the above interpretation of the biphasic nature of Rb release, only the f site would be available for inward transport under conditions of Rb–Rb exchange. And since only one ion binding site for external Rb^+ is involved, the dependence of Rb–Rb exchange on external Rb should be simply hyperbolic, which is just what was found in recent experiments by Karlish and Stein (1985).

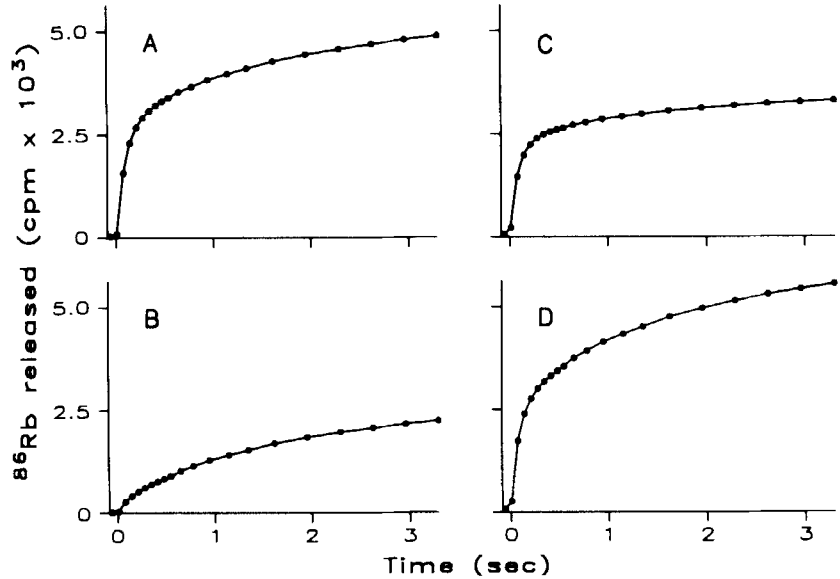

FIG. 10. Release of ^{86}Rb from the occluded state in the presence of $Mg^{2+}P_i$ and K^+. (A) Control conditions. (B) s site, early ^{86}Rb—the Na^+,K^+-ATPase was incubated with ^{86}Rb followed by a 2-sec incubation with unlabeled Rb and $Mg^{2+}P_i$ at 5°C and then returned to buffer, followed by the time course in K^+ $Mg^{2+}P_i$. (C) f site, late ^{86}Rb—the Na^+,K^+-ATPase was incubated with unlabeled Rb followed by a brief incubation with ^{86}Rb and $Mg^{2+}P_i$ and then returned to buffer, followed by the time course in K^+ $Mg^{2+}P_i$. (D) Algebraic sum of C and D.

VI. CONCLUSION

It is clear that the mechanism by which the Na^+,K^+-ATPase is able to use chemical energy to drive the transmembrane movements of Na^+ and K^+ ions is by no means solved, despite decades of careful investigation. Many of the gaps in our knowledge are related to the nature of the ion binding sites and their interactions—even the question remains open as to whether the sites for Na^+ and K^+ are distinctly different sites or whether there is one set of sites with different specificities depending upon enzyme conformation. The studies summarized here are the beginnings of an attempt to ascertain the temporal relationships of ion binding, translocation, and release and to place these events in the cycle of nucleotide binding–enzyme phosphorylation–dephosphorylation.

The present results on the movements of Na and K in a single turnover of the Na/K pump strongly support the conventional model of ion transport (Fig. 1) in which Na movement is an early event in the turnover of the pump following binding of ATP, and K movement is later in the cycle and involves the major rate-limiting step. Certainly it would not be easy to reconcile these results with models in which both Na and K ions were simultaneously transported. As to whether at one point in the cycle both Na and K ions might be simultaneously bound to separate transport sites on the extracellular face of the pump, the results are not yet convincing: While our data on ^{22}Na movement in dog kidney vesicles and ^{86}Rb movement in eel electroplax vesicles suggest overlap of site occupancy, the possibility that this is an artifact due to a species difference remains to be investigated.

Measurements of the time course of dissociation of tightly bound ^{86}Rb from the Na^+,K^+-ATPase have demonstrated that the proposed occluded state is kinetically competent to be included in the cycle of Na/K pumping driven by ATP, as well as in the partial reaction of Rb–Rb exchange promoted by P_i and nucleotide; in agreement with earlier studies of others, the properties of the occluded state are found to be those expected of such an intermediate. Observation of the time course of ^{86}Rb dissociation in the presence of $KMgP_i$ has revealed two distinct binding sites for K^+ in the occluded state, giving an excellent indication of the potential value of examination of rapid ion movements.

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Chapter 3

Properties of Epithelial Potassium Channels

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I. K CHANNELS: UNITY AND DIVERSITY

In this chapter, I will attempt to review some of the properties of K channels in epithelial cells. I will concern myself not only with what we know, but also with what we might expect as studies of epithelial ion channels progress. I will emphasize one particular expectation for epithelial K channels, namely, diversity. The small amount of data now available suggests that we should expect that different K-related cell functions or responses may be served by distinct populations of K channels which can be differentiated according to their physical properties. I will discuss briefly the general properties of ion channels and emphasize the experimental strategies which can be employed to differentiate one type of K channel from another. Excellent surveys of the ion channels in epithelial cells are found in articles by Lewis *et al.* (1984a) and Van Driessche and Zeiske (1985).

A. General Properties of Ion Channels

Our expectations for ion channels have been particularly shaped by the development of techniques which permit the properties of single ion channels to be studied directly. These techniques have altered our consciousness to the point where it now seems possible to use the label ion channel without being subjected to endless philosophical debates about the status of such transport elements in reality.

To begin, we consider briefly some of the properties which we might expect all ion channels to have. It is useful to group these properties under two headings: conduction and gating (Latorre and Miller, 1983). Conduction properties are those which govern the rate of ion translocation through an open channel, while gating properties are those which determine whether the channel is in a conducting or a nonconducting state.

Ion conduction is usually depicted as occurring via macromolecules which span the lipid matrix of the membrane. From the standpoint of ionic permeability, the function of such channels is to provide a local environment of relatively high dielectric constant which allows a small charged particle to overcome the electrostatic energy barrier associated with leaving the very polar aqueous environment adjacent to the membrane. Studies of the effect of temperature on ionic conductance suggest an activation energy similar to that for aqueous diffusion so that the notion of a water-like pore may be a reasonable first approximation (Hille, 1984).

The ion channels studied in detail so far are purely dissipative elements. Ion flows are driven by transmembrane electrochemical potential gradients, $\Delta\tilde{\mu}_i$, where

$$\Delta\tilde{\mu}_i = RT \ln(C^i{}_1/C^i{}_2) + zFV_m$$

where R, T, z, and F have their usual significance, and V_m, C_1, and C_2 are the membrane potential and the ion concentrations on the two sides of the membrane, respectively. Ion channels reduce the barriers to transmembrane ion flow sufficiently that electrochemical gradients of the order of 100 mV can produce ion flow of 10^6–10^8 ions/sec through a single channel! These high rates of translocation are an identifying feature of channel-mediated ion flows.

The study of ion channels by fluctuation analysis (Anderson and Stevens, 1973) and single-channel techniques (Hamill *et al.*, 1981) reveals that a general property of ion channel molecules is that they undergo conformational transitions between conducting (open) and nonconducting (closed) states. This probabilistic behavior is revealed by the fluctuations in the macroscopic current through an ensemble of channels and in the unitary openings and closings which are by now familiar in single-channel records. The existence of the stochastic component to ion channel behavior places another requirement on any description of ion flow through an ensemble of channels: It is necessary to recognize that the total ion flow depends not only on the conducting properties of individual open channels, but also on how many are open at any given instant. This principle is particularly important in any study of the modulation or regulation of ion flows. Ionic currents across epithelial membranes are subject to various and subtle types of regulation. In the case of the effect of aldosterone on active Na absorption, for instance, the stimulatory effect of the mineralocorticoid could be detected as an

increase in short-circuit current due to active Na absorption (Sharpe and Leaf, 1966), and early studies focused on the question of change in the macroscopic Na conductance of the apical membrane (Civan and Hoffman, 1971). Fluctuation analysis, however, revealed that apical Na conductance increased due to an increase in the number of Na channels (Palmer *et al.*, 1982). Thus, in general, we must distinguish between two types of regulation: simple gating of the channel, i.e., changing the probability of finding the open state (mean number of open channels), and changes in the number of channels in the membrane due to the insertion or retrieval of membrane protein.

B. Current Voltage Relations: From Single Channels to Macroscopic Conductance

The most economical summary of the properties of an open ion channel is the current–voltage relation. Since the channel is a purely dissipative element, the simplest approach is to take the driving force for ion flow to be the difference in electrochemical potential, $\Delta\tilde{\mu}_i$. To obtain the current–voltage relation, we convert ion flow to units of current using the relation

$$I_i = z_i F J_i$$

where I_i is the current due to the flow of the ion, i, in amperes per square centimeter, J_i is the flow of the ion, i, in moles per square centimeter per second, and z_i and F have their usual meaning.

The total driving force, $\Delta\tilde{\mu}_i$, can be converted from the fundamental units of joules per mole to the electrical units of volts (joules per coulomb), i.e.,

$$\Delta\tilde{\mu}_i/zF = (RT/zF)\ \mathrm{In}(C^i_1/C^i_2) + V_m$$

or, more conventionally,

$$\Delta\tilde{\mu}_i/zF = E_i - V_m$$

where E_i is the ionic electromotive force (Nernst or equilibrium potential) defined as $E_i = (RT/zF)\ \mathrm{In}(C^i_1/C^i_2)$. The current–voltage relation is then obtained by defining the ionic conductance, γ_i, by the relation

$$I_i = \gamma_i(E_i - V_m)$$

This current–voltage relation is a statement of Ohm's law for a single ion channel. The conducting properties of the open channel are contained in the value of γ_i. The value of γ_i will depend on the permeability of the channel to the ion in question, i.e., the ease with which a particular ion can enter the channel, translocate, and exit on the other side. The term permeability in this context may

be equated with that which would be measured in a hypothetical tracer flow measurement through a single channel which is always open. In general, however, the single-channel conductance will also depend on the ambient ion concentration, since the local availability of ions will determine the probability of finding an *open* channel in an occupied (conducting) state. To appreciate this point, it is necessary to realize that a single-channel current (see also Section III) measures the *time average* conductance of the open channel. If a current of 1 pA (10^{-12} amperes) flows for 1 sec, this represents the movement of about 10^6 ions. Thus, the amount of current per unit driving force (i.e., the conductance) will reflect not only the permeability of the open channel, but the probability of finding an ion in it (Latorre and Alvarez, 1981; Latorre and Miller, 1983).

The single-channel conductance defined in this way is most unambiguous when the channel is bathed by identical concentrations of the permeant ion on both sides. In the presence of gradients of the permeant ion, the I–V relation may exhibit rectification of the type described by the Goldman equation (Goldman, 1943).

To extend this description to macroscopic currents, we must introduce formally the probabilistic behavior of the channel; i.e., it is necessary to account for the fact that the channel is not in a conducting state all of the time. We define the macroscopic ion current as

$$I_i = \Sigma i_i = g_i\,(E_i - V_m)$$

where i_i is the single-channel current and g_i is the macroscopic conductance. The macroscopic conductance, g_i, is related to the single-channel conductance by the relation

$$g_i = \gamma_i <N_i> = \gamma_i P_o N_i$$

where $<N_i>$ is the mean number of open channels, N_i is the total number of channels (counting open and closed), and P_o is the probability of finding an open channel. The macroscopic current–voltage relation may then be rewritten as

$$I_i = (\gamma_i P_o N_i)\,\{E_i - V_m\}$$

This equation summarizes concisely our expectations for ion channels in epithelial cells. These channels will mediate the flow of ion currents across epithelial cell membranes due to imposed electrochemical potential gradients. The magnitude of the ion current will depend on four factors: (1) the total driving force, (2) the total number of channels, (3) the probability of finding a single channel in a conducting state, and (4) the conductance of the open channel. This relation also constitutes an analytical framework within which we may view the modulation or regulation of ion translocation in terms of these four parameters.

II. METHODS FOR STUDYING ION CHANNELS

This section will review briefly some of the techniques employed to determine the properties of epithelial ion channels in both a macroscopic and microscopic setting.

The properties of ion channels can be inferred from a variety of measurements of the electrical properties of epithelial cells, some of which are summarized in Fig. 1. I will consider here only methods in which cellular ionic currents are measured more or less directly. It should be emphasized, however, that much of our understanding of epithelial membrane conductances derived from studies of intracellular *potential*. Determination of membrane conductance from such measurements, however, depends on the use of equivalent circuits which must em-

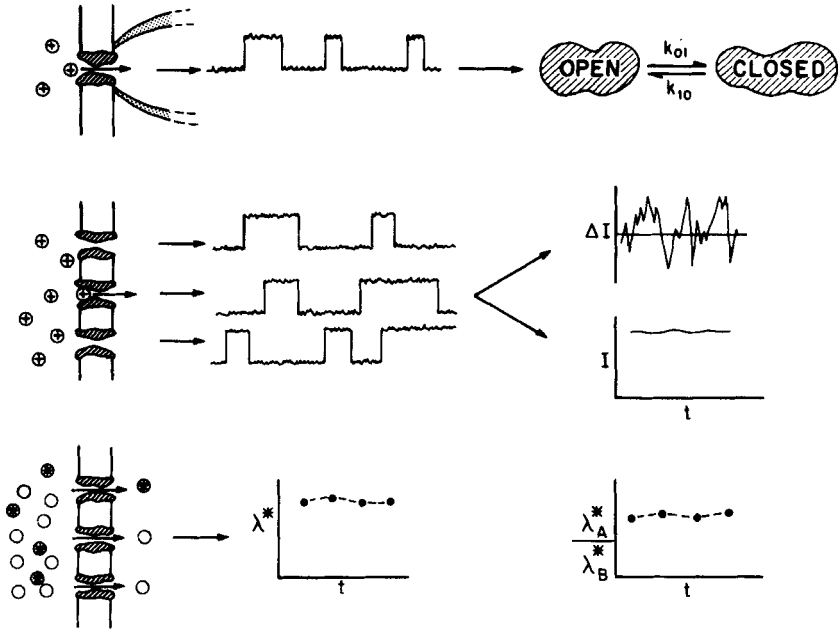

FIG. 1. Methods for studying ion channels. Top: Single-channel techniques—patch clamping and reconstitution—clearly differentiate the conduction properties from the gating properties of a channel. Middle: The total macroscopic current, I, through an ensemble of channels is a time average measurement in which the distinction between conduction and gating is lost. Fluctuations, ΔI, in the macroscopic current are a result of the random behavior of the underlying single channels. Analysis of the fluctuations can provide an estimate of the size and time course of the underlying single-channel event. Bottom: Tracer rate coefficients, λ^*, also provide a time average measure of the conduction–gating properties of an ensemble of channels. Tracer flux ratios, λ^*_A/λ^*_B, however, are independent of gating.

brace all of the complexities of epithelial structure. Conductances are obtained from cable analysis or resistance perturbation methods. A discussion of these methods is beyond the scope of this chapter. Likewise, the measurement of transepithelial impedance can, in principle, provide information about cell membrane conductance in a complex structural context. Again, however, this method requires that rather elaborate equivalent circuits be employed to extract the parameters. Interested readers are referred to excellent reviews of these techniques (Diamond and Machen, 1983; Petersen and Findlay, 1982; see also Wills *et al.*, this volume).

A. Macroscopic Ionic Currents in Single Membrane Preparations

The study of the ionic currents across the membrane of the squid giant axon by Hodgkin and Huxley (1952) exemplifies the classic approach to the study of ion channels. One landmark contribution of this work was the demonstration by Hodgkin and Huxley that it was possible to identify and quantify ionic currents due to specific ions, i.e., Na and K. These ion-specific Na and K currents have been clearly separated on the basis of voltage dependence, ion selectivity, and pharmacological specificity, and clearly represent different populations of ion-specific channels in the axon membrane (Hille, 1984).

The earliest measurement of ionic currents across epithelial membranes, those of Hans Ussing and colleagues (Ussing and Zehran, 1951; Koefoed-Johnsen and Ussing, 1958), were already compromised by the series arrangement of epithelial membranes. A primary goal of later studies has been to determine the unique properties of apical and basolateral membranes. One approach to this problem has been the use of a variety of techniques to functionally eliminate either the apical or the basolateral membrane as a barrier to ion flow, so that the properties of the other membrane may be measured more or less directly.

Studies of the apical membrane of frog skin (Fuchs *et al.*, 1977) and toad bladder (Palmer *et al.*, 1980) have been conducted by using potassium Ringer's solutions on the serosal side to depolarize the basolateral membrane potential and increase basolateral conductance such that the transepithelial potential is equal to the apical membrane potential. This method permits the "instantaneous" I–V relation of the apical membrane to be measured. The use of this method requires that the assumption of basolateral depolarization be validated independently.

A second approach is the use of pore-forming antibiotics to increase the conductance and reduce the cation selectivity of the apical or basolateral membranes. The most popular of these pore formers are the polyenes; amphotericin (Lichtenstein and Leaf, 1965; Kirk *et al.*, 1980), nystatin (Lewis, *et al.*, 1977; Wills *et al.*, 1979), and filipin (Neilsen, 1979) have been employed. These

techniques all exploit the fact that the polyenes, when added to one side of a biological or artificial membrane, form pores (Marty and Finkelstein, 1975), which are moderately selective for cations over anions ($P_{Na}/P_{Cl} \approx 7$), but which do not discriminate among univalent cations. This method has been employed to eliminate the apical membrane as a barrier to cation flow in a variety of tight epithelia. In addition, Palmer *et al.* (1980) and Benos *et al.* (1983) used polyenes to increase the cation permeability of the basolateral membranes of toad urinary bladder and frog skin, respectively. Another antibiotic, gramicidin D, has been employed to reduce apical membrane resistance in rabbit urinary bladder (Lewis and Wills, 1982). Figure 2 illustrates the paradigm used by Kirk *et al.* (1980) and Germann *et al.* (1986a) to measure K currents across the basolateral membranes of colonic epithelial cells. In the presence of a transmural K gradient, the addition of amphotericin B to the mucosal bath induces a transcellular current due to the movement of K down an electrochemical potential gradient from mucosa to serosa. This method for the measurement of basolateral K currents has several

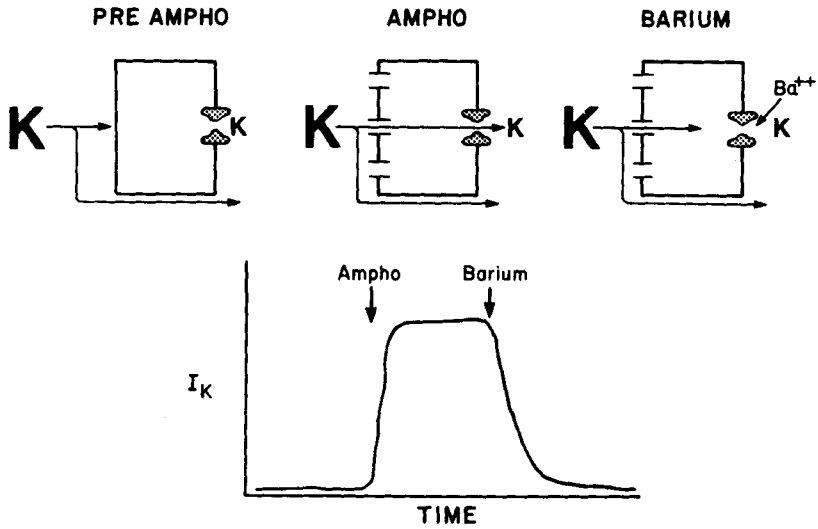

FIG. 2. The use of pore-forming antibiotics to "functionally isolate" basolateral membranes of tight epithelia. Transmural K gradient is imposed across an epithelial cell layer mounted in an Ussing chamber. Short-circuit current (I_{sc}) is low due to high resistance and relative nonselectivity of shunt path and relative impermeability of apical membrane to K. Addition of polyene (amphotericin B or nystatin) to the mucosal bathing solution results in formation of cation-selective pores in the apical membrane. Permeabilizing the apical membrane leads to the generation of a transcellular K current due to the K selectivity of the basolateral membrane. Addition of a K channel blocker (barium) reduces K current. Basolateral K conductance is operationally defined as $g^K_{bl} = I_K/E_K$. Note that the use of short-circuit conditions reduces "leakage" currents via shunt pathways. Modified from Germann (1984).

advantages. In the presence of high mucosal K, the polyene-induced conductance is more than an order of magnitude greater than the native basolateral membrane conductance (Kirk and Dawson, 1983), so that the K currents are determined by basolateral membrane conductance. The polyene-induced conductance appears to be relatively stable in the presence of mucosal polyene, but it is also reversible. The apical permeability change is also relatively specific for monovalent cations; calcium permeability does not appear to be altered. The polyene effect appears to be restricted to the apical membrane; i.e., polyene molecules do not appear to cross the apical membrane, enter the cell, and insert in the basolateral membrane. Inhibitors of basolateral K currents do not block polyene-mediated apical K entry, and the basolateral membrane retains a high selectivity for K over Na (Kirk and Dawson, 1983; Germann *et al.*, 1986a).

The polyene-treated cell layer permits the measurement of steady-state ionic currents under short-circuit conditions ($V_m = 0$). The use of short-circuit conditions has the important advantage of producing ionic currents only through pathways characterized by high ionic selectivity. In the turtle colon, for instance, a substantial K gradient across the shunt path produces very little current due to the low ion selectivity of this path (Fig. 2). The steady-state currents may be compared with tracer fluxes. This not only facilitates the unambiguous identification of the current-carrying ions, but also permits the determination of transport kinetics. The polyene-induced change in apical membrane permeability also facilitates certain modifications of intracellular composition, i.e., Na and K content or intracellular pH (Eaton *et al.*, 1984).

The ionic currents measured in polyene-treated tissues are macroscopic currents and hence suffer from the same disadvantages of all such measurements: Changes in current can be caused by changes in the number of conducting channels as well as the properties of individual channels. More problematic, however, is the effect of cellular heterogeneity which could complicate the interpretation of the ionic current through many cells in parallel.

The selectivity of the polyene-induced permeability is an advantage in that it enables the cell to retain most of its constituents despite high permeability to monovalent cations. In view of the ubiquitous role of Ca^{2+} as an intracellular mediator, however, it would be desirable to be able to readily modify the intracellular concentration of this ion. Chang and Dawson (1985) used digitonin, a detergent, to permeabilize the apical membrane of turtle colon to calcium and monovalent cations. Using this preparation, it was possible to show that a rise in cellular calcium activated conductances for K and Cl in the basolateral membrane (Chang and Dawson, 1985, 1986).

B. Whole-Cell Recording

The introduction by Neher and his colleagues (Neher and Sakmann, 1976; Hamill *et al.*, 1981; Sakman and Neher, 1983) of techniques for forming tight

electrical seals between glass pipettes and cell membranes has had the revolutionary effect of making voltage clamp studies of ionic currents applicable to virtually any cell. In this recording configuration, a gigaohm seal (see below) is formed between the tip of a fire-polished pipette and a cell. Then the patch of membrane within the pipette is ruptured. This permits the total membrane current to be measured directly and allows equilibration of the cell interior and the pipette-filling solution. This arrangement permits the cell membrane conductance to be determined over a wide voltage range under conditions where the intracellular and extracellular milieu can be precisely controlled. In addition, it is possible to obtain single-channel measurements in the whole-cell configuration if the number of open channels is reduced.

C. Fluctuation Analysis

All macroscopic current measurements suffer from the same fundamental limitation: The distinction between conduction and gating is obscured. The macroscopic current is a function not only of the properties of an individual channel, but also the number which are open. Fluctuation analysis can be employed to resolve these two important properties of channels in macroscopic currents. Anderson and Stevens (1973) showed that it was possible to measure the fluctuations or "noise" associated with macroscopic currents through an ensemble of ion channels and showed how to use the fluctuations to estimate the properties of single ion channels. Lindemann and Van Driessche (1977) showed that it was possible to apply this technique to macroscopic currents across sheets of epithelial cells of the frog skin. They were able to resolve the fluctuations due to the reversible blockade of apical sodium channels by amiloride and thus provided the first estimate of single-channel properties in an epithelium.

Fluctuation analysis is based on the notion discussed above that a macroscopic ionic current represents the average value of the sum of many single-channel ion currents. Each single-channel current is constantly fluctuating between at least two states: $i = 0$ (channel closed) and $i = i_i$ (the open-channel current). This random switching behavior of individual channels gives rise to fluctuations in the macroscopic current because the total number of open channels may vary widely from moment to moment, although the time average value is constant. Single-channel currents in the picoampere range cause fluctuations in macroscopic currents which are in the nanoampere range and can be resolved on an oscilloscope using low-noise electronic circuits (Van Driessche and Gullentops, 1982; Van Driessche and Lindemann, 1978). The object of the analysis is to extract the magnitude and the periodicity of the fluctuations, parameters which can be related to the underlying single-channel events, i.e., the single-channel currents and the mean open time. Most commonly this is done by subjecting the data to a fast Fourier transform. The Fourier transform may be thought of as measuring the frequency composition of the fluctuations. If the mean open and

closed times are short, the fluctuations will be dominated by high-frequency components. If the mean open and closed times are long, low frequencies will dominate the noise. The result is expressed on a log-log plot called the power spectral density or power spectrum in which a function of the square of the variation in current is plotted versus frequency. The desired result is a plot such as that shown in Fig. 3a which can be described by a Lorentzian-type function of the form

$$S(f) = S_0/[1 + (f/f_c)^2]$$

where $S(f)$ is the power spectra density in $(\text{amps})^2 \cdot \text{sec}/\text{cm}^2$; f the frequency in Hz; S_0 the plateau value at low frequencies; and f_c the corner frequency where $S(f_c) = S_0/2$.

A spectrum of the Lorentzian form is the ideal result of fluctuations due to a single time constant relaxation process, i.e., one in which the underlying event is the random switching of channels between two states: open and closed.

$$\text{Open} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{Closed}$$

where α and β are the rate coefficients which describe the rates of the conformational changes. The shape of the Lorentzian curve is completely characterized by two parameters: the low-frequency plateau, S_0, and the corner frequency, f_c. The corner frequency is directly related to the intrinsic rate for the open-to-closed transition by

$$2\pi f_c = \alpha + \beta$$

Similarly, the plateau value S_0 is related to the total number of channels by

$$S_0 = 4N_i^2 P_o P_c / 2\pi f_c$$

where P_o and P_c are the probabilities of finding an open and closed channel, respectively; N is the total number of channels; and i the single-channel current. In practice, however, data from epithelial tissues are usually described by the sum of *two* components (Fig. 3b and c), a Lorentzian component and a component which varies inversely with the frequency. The latter is referred to as $1/f$ noise (Fig. 3b), and its origin is not well understood (see Lewis *et al.*, 1984a; Van Driessche and Gullentops, 1982).

In many instances, it is not possible to extract a Lorentzian component from the spontaneous fluctuations, but it is possible to induce a Lorentzian component in the power density spectrum by applying a reversible blocker of the channel. The blocker creates a two-state, exponential relaxation process by continually binding and unbinding from the channel. For example, a Lorentzian component in the sodium current noise from frog skin was only detectable after the addition

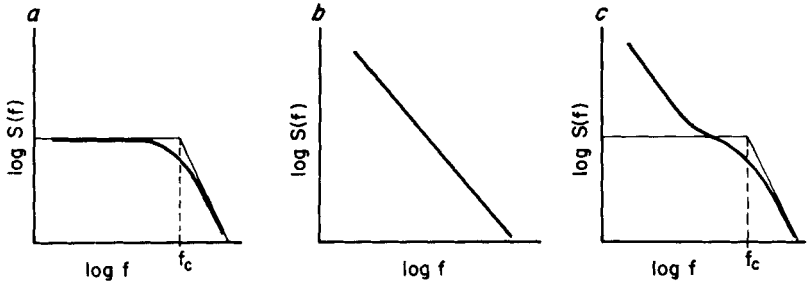

FIG. 3. Ideal power density spectra obtained by analyzing fluctuations in macroscopic currents using Fourier transform methods. (a) Ideal Lorentzian spectrum due to a single population of two-state channels, each of which is gated randomly. (b) "$1/f$ noise" typically observed in spectra from epithelial tissues. (c) Sum of a single Lorentzian and a component of $1/f$ noise.

of the reversible blocker, amiloride (Lindemann and Van Driessche, 1977). Accordingly, it is observed that the corner frequency of the blocker-induced Lorentzian, a measure of the frequency of the on–off event, is increased as the concentration of the blocker is increased. Lindemann and Van Driessche showed that this behavior can be interpreted in terms of a simple two-state model (1977) where

$$2\pi f_c = k_{01}[B] + k_{10}$$

$[B]$ is the blocker concentration and k_{01} and k_{10} are the rate coefficients for the on and off reactions, respectively.

The linear relation between corner frequency and blocker concentration described by the equation above is of practical significance because it permits an estimate of the values of k_{01} and k_{10}. Values for these rate coefficients lead directly to a calculation of the open and closed probabilities, P_o and P_c, and thus permit a calculation of the single-channel current, i, and number of channels, N. Thus, blocker-induced fluctuations can lead to an estimate of channel density and, if the electrochemical driving force is known, to a calculation of the single-channel conductance (Lindemann and Van Driessche, 1977). This information cannot, in general, be extracted from an analysis of spontaneous fluctuations because the probabilities, P_o and P_c, are not known.

D. Single-Channel Measurements

1. Patch–Clamp Studies

The patch–clamp technique permits the resolution of the conducting behavior of a single ion channel in a cell membrane (Hamill *et al.*, 1981; Sakmann and Neher, 1983). The power of the technique is further enhanced by the ability to

study membrane patches either on the cell or detached from the cell so that either the cytoplasmic side or the extracellular side of the membrane faces the bath. Thus, the technique offers not only unprecedented resolution, but also unprecedented control of the environment of the channel.

The patch–clamp technique has already been applied to a variety of epithelial preparations, including the epithelium of the lens (Rae and Levis, 1984), pancreatic acinar cells (Maruyama and Petersen, 1982), kidney tubules (Koeppen *et al.*, 1984; Palmer and Frindt, 1986; Gögelein and Gregor, 1984), tubules of the shark rectal gland (Greger *et al.*, 1985), urinary bladder cells (Hanrahan *et al.*, 1985), cultured cell lines (Nelson *et al.*, 1984; Hamilton and Eaton, 1985), and dissociated colonic cells (Richards and Dawson, 1986a,b).

2. Reconstitution

From a biophysical point of view, perhaps the ideal way to study the properties of individual channels is to use so-called reconstitution methods [see Miller (1986) for an excellent summary]. The aim of such techniques is to "extract" the channel protein from a membrane preparation and then insert the channel protein into a planar bilayer in such a way as to preserve or "reconstitute" its conducting properties (Miller, 1983; Coronado and Labarca, 1984). In this ideal setting, the channel can be studied under conditions where the measurement of ionic currents and gating is relatively easy while the environment of the channel may be exquisitely controlled. This attractive endpoint is difficult to reach, however, due to problems inherent in obtaining channel preparations which are relatively pure and which will form conducting units in bilayers. At this point, at least two reconstitution attempts have been successful using epithelial membranes as starting material: the apical Na channel from A6 cells (Sariban-Sohraby *et al.*, 1984) and a K channel from olfactory epithelium (Vodyanoy and Murphy, 1983).

III. K CHANNEL DIVERSITY: "FINGERPRINTS" FOR ION CHANNELS

It is now apparent that one general feature of K channels is diversity. The review by Latorre and Miller (1983) lists six types and Rae (1986) and Rae and Levis (1984) report at least six within a single lens epithelial cell. Even in the absence of a great deal of supporting evidence, this diversity of channel types creates the expectation that different cell functions (or responses) which involve alterations in cell membrane K permeability may be subserved by different populations of K channels which are activated or inactivated under a variety of circumstances. I will provide several examples from our own laboratory which

suggest that the epithelial cells of the turtle colon, a relatively simple Na-absorbing epithelium, can exhibit several different types of K conductance. First, however, I will consider briefly the criteria for identifying different types of channels.

A. Unitary Conductance and Kinetics of Ion Conduction

The most widely used and readily available parameter for characterizing ion channels is the unitary or single-channel conductance. This parameter is obtained directly in a single-channel experiment (patch–clamp or bilayer) from the slope of the single-channel I–V relation. As discussed above, the most unambiguous measure of this conductance is obtained under conditions where the ionic concentrations are identical on both sides of the membrane. It is important to recognize, however, that the single-channel conductance is not solely a property of the ion channel. As discussed above, the value is expected to be highly dependent on the concentration of the permeant ion. Furthermore, the conductance may be a saturable function of ion concentration. It is instructive to consider the origin of this saturable single-channel conductance for a channel which can only contain one ion. I will essentially follow the treatment of Lattore *et al.* (1981, 1983). The saturation of single-channel conductance for a channel which can *only* contain one ion at a time is comprehensible if it is recognized that a single-channel current represents a time average measurement; i.e., during a 1 pA, unitary step of current of duration 1 sec, 10^6 ions move through the channel. The single-channel conductance will be a function of two processes: one determining how readily ions enter the channel, and the other determining, once in the channel, how easily they exit. For a single-ion channel, these two processes are connected by the constraint that an ion cannot enter an occupied channel. If Θ is the probability that the channel is *occupied* then the rate of entry and hence the conductance, γ, will be proportional to the ion concentrations, a rate coefficient, β, which describes the entry process, and the probability of finding the channel *unoccupied*, $1 - \Theta$,

$$\gamma = \beta[K]\,(1 - \Theta)$$

The conductance of an *occupied* channel must be inversely proportional to the mean transit time, T, i.e.,

$$\gamma = A\Theta/T$$

where A is a constant. Combining these two relations yields a relation for γ as a function of $[K]$, which has the form of the Michaelis–Menten equation

$$\gamma = (A/T)\,[K]/\{(A/\beta T) + [K]\}$$

Saturation is thus envisioned as arising from the limitation on the average translocation rate imposed by the rate of exit of the occupying ion from the channel. Saturation occurs when the residence time is so long that raising the ion concentration cannot increase the rate of entry, since virtually all of the channels are already occupied. For multiply occupied channels, even more complex relations between conductance and ion concentration may be expected (Hille and Schwarz, 1978).

In principle, single-channel conductances may be obtained from measures of current fluctuations, although these estimates are highly dependent on obtaining a reasonably homogenous population of channels and on the utilization of a specific model to interpret the spectral data (Stevens, 1984; Van Driessche and Gullentops, 1982; Lewis *et al.*, 1984a).

B. Ion Selectivity

It has long been recognized that ion selectivity is an important aspect of cell function. The change in the ion selectivity of the axon membrane was a key to understanding the nature of the action potential (Hodgkin and Huxley, 1952) and the recognition by Koefoed-Johnson and Ussing (1958) of the difference in the ion selectivity of the apical and basolateral membrane of epithelial cells was a key to formulating a realistic model of transepithelial Na transport. The development of techniques for determining the properties of single-ion channels directly has served to underscore the notion that ion channels can differ widely in their ability to discriminate between ions, so that ion selectivity becomes an important component in the "fingerprint" (Latorre and Miller, 1983) for identifying an ion channel. We recognize channels which are specific for particular cations, Na and K channels, for instance, and others which appear to select purely on the basis of valence (Yellen, 1982).

Ion selectivity is usually judged by comparison with a baseline sequence based on the mobility or conductance of ions in free solution where ionic migration may be regarded, at least to a first approximation, as a hydrodynamic problem having to do with the difference in the hydrated radii of different ions (Hille, 1984). Accordingly, the sequence of free solution mobilities for alkali metal cations is $Cs \approx Rb > K > Na > Li$, the inverse order of the atomic radii, but in order of increasing hydrated radius. The fact that the selectivity sequence displayed by ion channels differs widely from this is one clue that permeation through an ion channel differs from diffusion in water. The physical basis for ion selectivity is not well understood, a fact which is not surprising since the rate of ion permeation depends on the details of interaction of the ion with ligands in the channel. Eisenman's theory (Eisenman and Krasne, 1975) provides a general approach based on the relative difference between energies of hydration and

energies of Coulombic interaction with charged or polarizable sites in the channel. This relatively simple model predicts, in fact, most of the known biological selectivity sequences, the notable exception being the delayed rectifier of snail neurons studied by Reuter and Stevens (1980). What is most germane to this discussion, however, is that ion selectivity differs widely and thus offers an opportunity for a very sophisticated fingerprint for an ion channel.

To understand ion selectivity measurements, it is necessary to examine the significance of the term permeability in terms of single-channel properties. In particular, it is imperative that the concept of single-channel permeability be clearly differentiated from the more nebulous notion of membrane permeability. Consider a hypothetical experiment in which the unidirectional flow of tracer K is measured across a membrane containing a single K channel. Let the channel be a single-ion channel which exhibits a saturable relation between the single-channel conductance and the K concentration, as discussed above, and let the channel be open continuously so that gating (i.e., opening and closing) does not affect the rate of tracer flow. The rate coefficient, λ^*, for tracer flow across the membrane is operationally defined by

$$\lambda^* = J^*/C^*$$

where J^* is the unidirectional, steady-state tracer flow and C^* is the tracer concentration on the "hot side."

Under what conditions will this tracer rate coefficient be a measure of the permeability of the open channel? Recall that even if, as in the present example, the channel is always open, it will not always contain a tracer ion; i.e., the tracer rate coefficient, like the single-channel current, is a time average measurement. In the presence of vanishingly small concentrations of unlabeled ion[1] the channel will rarely be occupied by an ion; thus, the rate of tracer flow will be determined by the physical properties which govern the processes of entry, translocation, and exit of tracer ion from the open channel. In this condition, the tracer rate coefficient measures the time average permeability of the channel. More importantly, for selectivity measurements the ratio of rate coefficients for two tracers, say ^{42}K and ^{86}Rb, would measure the ratio of the permeabilities for the two ions.

If the concentration of unlabeled ion is raised, however, the tracer rate coefficient would be reduced because now the open channel has a higher probability of being occupied by an unlabeled ion; i.e., the probability of finding the open channel unoccupied is reduced. The tracer rate coefficient is reduced due to the reduction in the rate of isotope flow, but clearly the permeability of the channel has not changed. In the presence of unlabeled ion, tracer ions experience the

[1] It is understood that the tracer ion is always present in amounts which are negligible on a molar basis (Dawson, 1982).

same resistance to permeation within the channel as in the absence of unlabeled ion, but the probability of finding an unoccupied channel has been reduced. Thus, we find that the apparent membrane permeability is reduced due to a reduction in the probability of finding the channel unoccupied, but the intrinsic channel permeability is unchanged. In our hypothetical experiment, this limitation can be overcome by measuring the ratio of the fluxes of two labeled ions, say ^{42}K and ^{86}Rb, in the presence of unlabeled K. Clearly, the ratio of the tracer rate coefficients would depend only on the ratio of the intrinsic permeabilities of the channel to the two ions, regardless of the ambient concentration of abundant K. The probability of the channel being occupied by abundant K would be the same for both. The situation is quite different, however, if the rate coefficients for the two isotopes are compared from two separate experiments using abundant Rb, on the one hand, and K, on the other (Hille, 1984).

From this perspective, it is clear that caution must be exercised in interpreting measures of selectivity. The values of the single-channel conductances in the presence of two different permeant ions, for instance, may not measure the ratio of the intrinsic channel permeabilities. The point is clear in the case of saturating conductance activity relations where saturation occurs at different ion concentrations (Hille, 1984).

Hille (1984) has shown that for channels which can be occupied by only one ion reversal potentials measure the intrinsic permeability ratio. This method has the advantage of yielding permeability ratios directly. In addition, for such channels, the measured permeability ratio should be independent of the absolute concentration of the ions and unaffected by saturation. In the case of saturable, one-ion pores, however, single-channel conductances and reversal potentials will not, in general, produce consonant results, since the former measures the average translocation rate while the latter measures the intrinsic permeability of an open channel. In the case of channels which can contain more than one ion at a time, the concept of permeability is even more difficult to define unambiguously, since it is necessary to differentiate between entry and translocation through single and multiply occupied channels (Hille and Schwarz, 1978; Hille, 1984). Here, for instance, mixed ion reversal potentials do not unambiguously define permeability ratios. In such cases, the conditions under which permeability is defined must be specified.

It will be obvious to the reader from the foregoing discussion that the problem of defining ion selectivity is further compounded when it is necessary to utilize macroscopic currents or tracer fluxes measured in an ensemble of channels, because in both of these measurements the distinction between conduction and gating is lost. Thus, selectivity ratios defined on the basis of macroscopic currents or fluxes are likely to reflect differences in the gating properties as well as the conduction properties of ion channels, i.e., exposing ion channels to gradients of rubidium rather than potassium might alter the mean open time of the

channels. One approach to this problem is to compare tracer flux ratios, since the ratios of the tracer fluxes will be independent of the mean open time.

C. Pharmacological Specificity and Gating

Although I will not dwell on this point, it is clear to anyone who has measured them that the success of many attempts to study ionic currents is critically dependent on the use of molecules which specifically inhibit or activate certain classes of ion channels. Some of these allow the separation of broad classes of channels; i.e., barium blocks many K channels (Kirk and Dawson, 1983; Latorre and Miller, 1983; Germann *et al.*, 1986). Others may, in some systems at least, exhibit specificity for one class of K channels. The bee venom apimin, for instance, has been found to be a specific blocker of a class of small (20 pS) calcium-activated K channels (Romey *et al.*, 1984). Germann *et al.* (1984, 1986a,b) found that quinidine and lidocaine specifically blocked K channels activated by cell swelling (see also below).

Similarly, the gating properties of a channel can serve to identify it, the most obvious example being voltage-dependent gating such as that observed in excitable membranes (Hille, 1984). Even in the absence of specific voltage or agonist-induced gating, the spontaneous gating of a channel, i.e., the mean open and closed times, or the amount of "flicker" (rapid brief closings) can distinguish one channel from another.

IV. TURTLE COLON: A MODEL SODIUM-ABSORBING EPITHELIUM

The large intestine of the freshwater turtle is a moderately tight, Na-absorbing epithelium (Dawson, 1977). The cellular mechanism of transmural Na transport is well described by the Koefoed-Johnsen–Ussing model originally developed for frog skin. As shown in Fig. 4, Na enters the cells across the apical membrane via Na channels which are blocked by amiloride. Na exit from the cell is mediated by an electrogenic pump which exchanges 3 Na for 2 K. Although there is a small amount of K secretion (Halm and Dawson, 1984a,b), most of the K which enters the cell appears to be recycled via the basolateral K conductance. The remainder of this discussion will focus on the basolateral K conductance of the turtle colon. Studies of the turtle colon suggest that conductive basolateral K exit can be mediated by at least two different types of K channels, which play different roles in the life of the cell.

I will present data from four types of studies which are representative of the approaches discussed above. First, I will discuss the measurement of basolateral

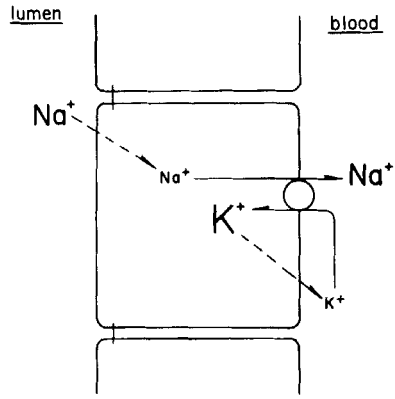

FIG. 4. The cellular mechanism of active Na absorption by turtle colon is adequately described by the Koefoed-Johnsen–Ussing Model: Sodium entry across the apical membrane via amiloride-sensitive Na channels and Na exit via an electrogenic pump which exchanges 3 Na for 2 K. The basolateral membrane cation conductance is dominated by K.

K currents in polyene-treated and digitonin-treated cell layers that provide the basic evidence for the diversity of K conductance of the basolateral membrane. I will then review some data from measurements of fluctuations in basolateral K currents which show how blocker-induced fluctuations might be used to separate out channel types. Finally, I will present data from patch–clamp studies of isolated colon cells which indicate that it is possible to track these channels through various levels of organization by their pharmacological specificity and ion selectivity.

A. Cholinergic Regulation of Na Absorption: Role of Basolateral K Conductance

We became interested in the role of the basolateral K conductance in cholinergic regulation of active sodium absorption because of results which are depicted in the composite shown in Fig. 5 taken from data of Venglarik and Dawson (1986). The dashed line shows the effect of carbachol on active Na absorption measured as the short-circuit current in the presence of Na Ringer's solution. Addition of the cholinergic agonist to the serosal bath caused a transient increase in I_{sc} followed by a profound inhibition. Tracer fluxes showed that the reduction in I_{sc} was due entirely to a reduction in active Na absorption. The response to carbachol was blocked by prior addition of atropine. The solid line in the figure shows the effect of carbachol on the basolateral K current measured in a polyene-treated colon as described earlier with potassium gluconate Ringer's

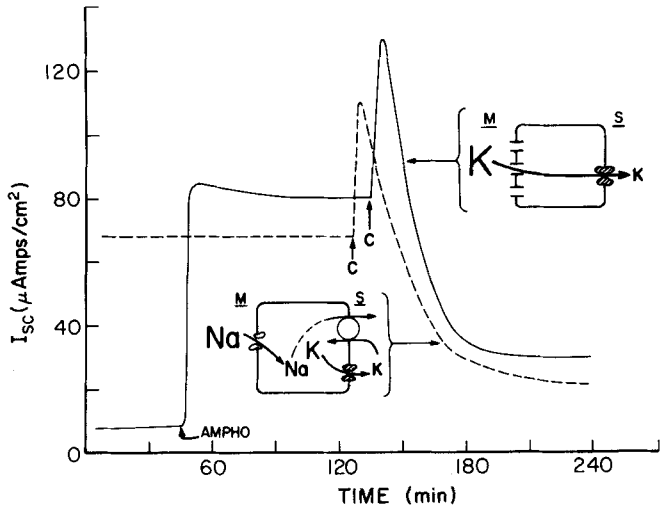

FIG. 5. Effect of cholinergic agonists on active Na absorption and basolateral K conductance. Dashed line: I_{sc} is a measure of active Na absorption as indicated in the inset. Addition of carbachol (10 μM, serosal bath) caused a transient increase in I_{sc} followed by a profound inhibition. Solid line: I_{sc} is a measure of basolateral K conductance as described in the text. Addition of carbachol (10 μM, serosal) produces a strikingly similar sequence of changes in g^{K}_{b1}, transient increases followed by profound inhibition. [Redrawn from original data of Venglarik and Dawson (1986).]

solution in the mucosal bath to prevent cell swelling. Serosal carbachol caused a transient increase in I_K followed by a profound inhibition. This response was also blocked by atropine. The striking similarity of the effect of the cholinergic agonists on active Na absorption and basolateral K conductance suggested that a cholinergically induced change in g^{K}_{b1} could be a primary event in the cholinergic inhibition of Na absorption. The response to a variety of agents demonstrated that the response is in both cases purely muscarinic (Venglarik and Dawson, 1986). In addition, both responses could be produced by maneuvers which lead to depolarization of the submucosal nerves (Venglarik and Dawson, 1986). If neostigmine was added to the serosal bath to prevent the hydrolysis of the acetylcholine, the addition of veratridine, an agent which depolarizes neurons by activating Na channels, produced the characteristic response in I_K and I_{Na}. This response was blocked by the addition of tetrodotoxin to the bath. It is interesting to note in this context that serosal barium can exert a dual effect on basolateral K conductance. Barium depolarized submucosal nerves and in the presence of neostigmine, produced a characteristic muscarinic response. Experiments with atropine-treated tissues showed, however, that the divalent ion also blocks the basolateral conductance directly.

B. Cell Swelling Activates a New K Conductance

The similarity between the responses of active Na absorption and basolateral K conductance to muscarinic agonists strongly suggests that the conductance measured as described above is the normal "resting" conductance of the epithelial cells which functions to "recycle" K as a part of Na absorption. This is the conductance measured in polyene-treated tissues when the mucosal bathing solution contains a potassium gluconate Ringer's solution and the serosal bathing solution is bathed by a regular NaCl Ringer's solution. Additional experiments, however, demonstrated that cell swelling caused an increase in basolateral K conductance. Furthermore, these experiments suggested that the effect of cell swelling was to activate a new population of K channels which did not contribute significantly to the resting conductance (Germann *et al.*, 1986b). We differentiated the resting and swelling-induced conductances on the basis of three criteria: pharmacological specificity, ion selectivity, and tracer kinetics (Germann *et al.*, 1986a,b). Figure 6 shows basolateral K currents produced as previously described with potassium gluconate in the mucosal bath and NaCl Ringer's solution

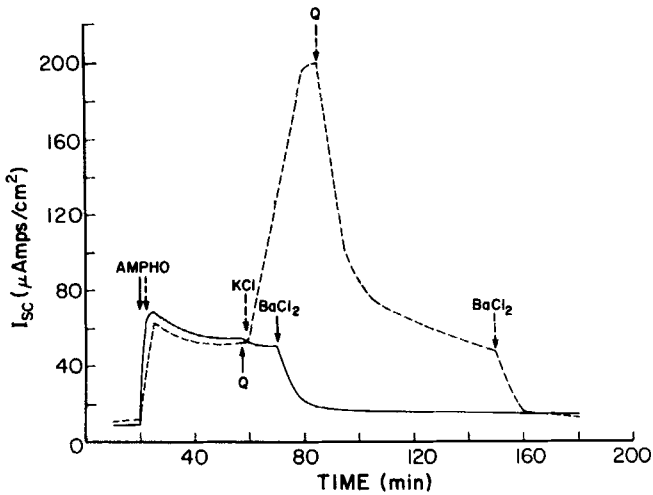

FIG. 6. Effect of cell swelling on basolateral K conductance. Solid line: Basolateral K current induced by mucosal amphotericin B under nonswelling conditions, i.e., potassium gluconate in the mucosal bath. Addition of quinidine (Q, 200 μ*M*, mucosal and serosal) has only a slight inhibitory effect, but the current is blocked by barium (5 m*M*, serosal). Dashed line: After induction of basolateral K current under nonswelling conditions, the mucosal bath was changed to KCl Ringer's to induce cell swelling. Cell swelling markedly increased I_K and the "extra" current was abolished by quinidine (Q), while the remainder was blocked by barium. The serosal solution for both tissues was sodium chloride Ringer's with atropine (10 μ*M*) to prevent effects due to release of endogenous acetylcholine.

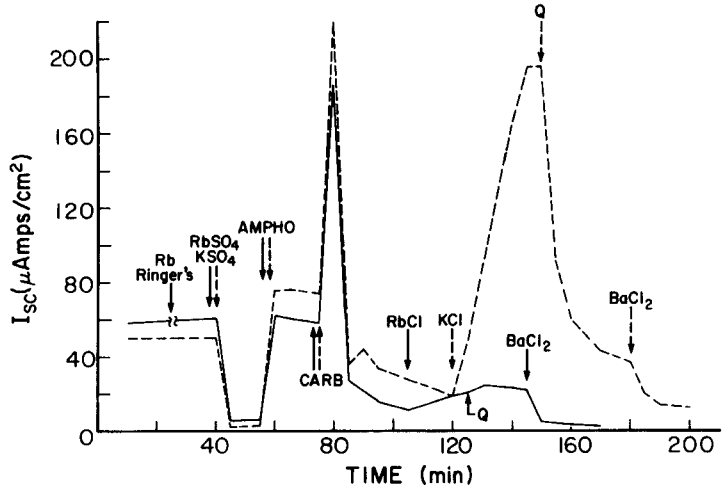

FIG. 7. Cation selectivity of the resting and swelling-induced basolateral K conductances. Two tissues were both initially bathed by NaCl Ringer's so that I_{sc} was a measure of active Na absorption. Changing one tissue to K-free, 2.5 m*M* Rb containing NaCl Ringer's (solid line) did not affect active Na absorption. Tissues were then exposed to transmural gradients of Rb (solid line) or K (dashed line) under nonswelling conditions (mucosal SO_4 + sucrose), and resting K or Rb currents were induced with mucosal amphotericin B. Currents due to K or Rb were of comparable magnitude and were both inhibited by carbachol. Subsequently, mucosal solutions were changed to RbCl (solid line) or KCl (dashed line) to induce cell swelling. A substantial quinidine-sensitive current was induced in the presence of mucosal KCl, but not in the presence of RbCl.

in the serosal. In this condition, the K current was virtually unaffected by quinidine, but was abolished by barium. In a second tissue, the mucosal bathing solution was switched from a potassium gluconate to KCl Ringer's solution. This treatment causes the epithelial cells to swell (Germann *et al.*, 1986b) due to the finite Cl permeability of the one-sided polyene pore and, as shown, markedly increased the K current. Most importantly, the extra current observed after swelling was specifically blocked by quinidine or lidocaine.

Figure 7 shows that currents measured under swelling and nonswelling conditions differ markedly in their cation selectivity. The figure shows that when a tissue was bathed by NaCl Ringer's solution under conditions of normal transport, this transport was unaffected by complete substitution of the extracellular K by Rb, whereas in tissues bathed by K-free solutions (not shown), the I_{sc} declined to zero, as expected due to the obligatory dependence of the basolateral Na/K pump on serosal K (Kirk *et al.*, 1980). Similarly, basolateral currents due to K or Rb can be induced by amphotericin B in the presence of transmural gradients of K or Rb under nonswelling conditions and currents due to either ion

exhibited the characteristic response to carbachol. Following carbachol inhibition of the resting conductance, the mucosal solutions were switched to chloride containing Ringer's solutions. This swelling condition induced a quinidine-sensitive basolateral K current in the presence of a K gradient, but virtually no current in the presence of an Rb gradient. Thus, under nonswelling conditions, it appeared that the basolateral membrane did not discriminate between K and Rb whereas the expression of the swelling-induced current was highly selective for K.

As discussed previously, any macroscopic definition of selectivity is of limited usefulness because of the lack of distinction between conduction through channels and the gating of channels in the presence of the substitute cation. Germann *et al.* (1986a) attempted to circumvent this problem by measuring, simultaneously, unidirectional fluxes of K and Rb in the presence of a K gradient under swelling and nonswelling conditions. Under swelling conditions, the ratio of the rate coefficient for M to S flow was $\lambda_K/\lambda_{Rb} = 2.6$, while $\lambda_K/\lambda_{Rb} = 1.5$ under nonswelling conditions. This result has two important implications. First, it confirms the notion that the basolateral membrane cation selectivity differs under swelling and nonswelling conditions. Second, the marked contrast between the ratio of the tracer rate coefficients in the presence of a K gradient and the ratio of the macroscopic K and Rb currents underlines the need to approach the issue of ion selectivity with caution.

To investigate the mechanism of K translocation via the quinidine-sensitive conductance, Germann *et al.* (1986a,b) measured bidirectional ^{42}K flux ratios in the presence of quinidine-sensitive (swelling) and quinidine-insensitive (resting) K currents. In the quinidine-sensitive condition, the ratio of the tracer rate coefficients was highly anomalous. Figure 8 shows an example of M to S and S to M ^{42}K rate coefficients in two paired tissues in which quinidine-sensitive basolateral K currents were induced in the M to S direction. The addition of the polyene induced a pronounced increase in the M to S rate coefficient. In contrast, the S to M rate coefficient was virtually unchanged despite the fact that the large K current indicated a significant K conductance. The behavior is expected for channels in which ions move by a single-file mechanism. In contrast, the ratios of bidirectional ^{42}K rate coefficients for the quinidine-insensitive (resting) K currents averaged around unity (Germann *et al.*, 1986a). The flux ratio analysis thus confirmed the general notion that the quinidine-sensitive and quinidine-insensitive currents were due to fundamentally different conduction processes.

Based on these results, we have formulated the following working hypothesis for the basolateral K conductance: In the normally transporting cell, basolateral K conductance is due to a population of resting K channels which are blocked by muscarinic agonists and by barium, but which are relatively insensitive to lidocaine or quinidine. Cell swelling activates a new set of channels blocked by quinidine and lidocaine as well as barium, which mediate rapid K exit from the cell, perhaps related to changes in cell volume.

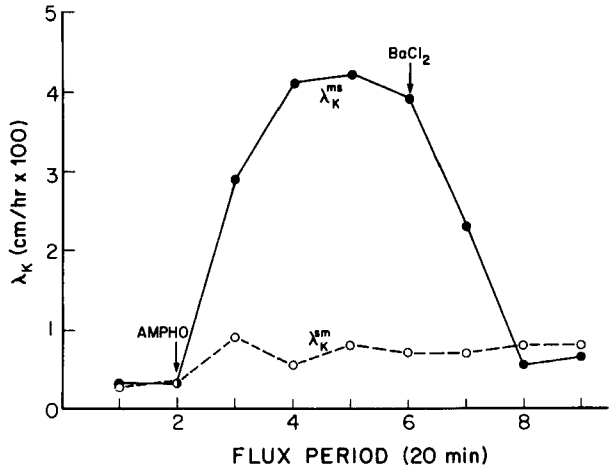

FIG. 8. Rate coefficients for mucosal to serosal (λ_K^{ms}) and serosal to mucosal (λ_K^{sm}) flow of ^{42}K in the presence of an M to S K gradient under swelling conditions. Prior to the addition of amphotericin B, rate coefficients were identical, since the majority of isotope flow occurred via noncellular (shunt) pathways. After amphotericin B, isotope flow in the direction of the K gradient (M to S) was favored by at least 5 to 1 over S to M flow. Serosal barium reduced the M to S rate coefficient to the level of the S to M rate coefficient. [Modified from Germann (1984).]

C. Are Calcium-Activated K Channels Involved?: Experiments with Digitonin

The sensitivity of swelling-induced currents to quinidine raised the possibility that this current might be due to potassium channels which were activated by a rise in cell calcium (Lew and Ferriera, 1978; Grinstein *et al.*, 1982). Calcium-activated potassium channels have been detected in many cells with use of the patch–clamp technique (Petersen and Maruyama, 1984). To circumvent some of the difficulties due to the uncertainty in cellular calcium levels, Chang and Dawson (1985) measured transmural K currents in tissues treated with digitonin to remove the apical membrane. This detergent has been employed to permeabilize cells to calcium (Dunn and Holz, 1983) and other large molecules. In these experiments, both sides of the tissues were bathed by chloride-free Ringer's with aspartate as the principal anion. The serosal solution was a sodium aspartate Ringer's solution which contained 1 m*M* calcium at pH 7.4. The mucosal solution was a potassium aspartate Ringer's solution which contained a Ca-EGTA buffer system at pH 6.6, adjusted such that the concentration of free calcium was about $10^{-9}M$. As shown in Fig. 9, under these conditions the addition of digitonin to the mucosal bath did not induce a K current, but subsequent addition of calcium to the low-calcium mucosal bath induced a current that

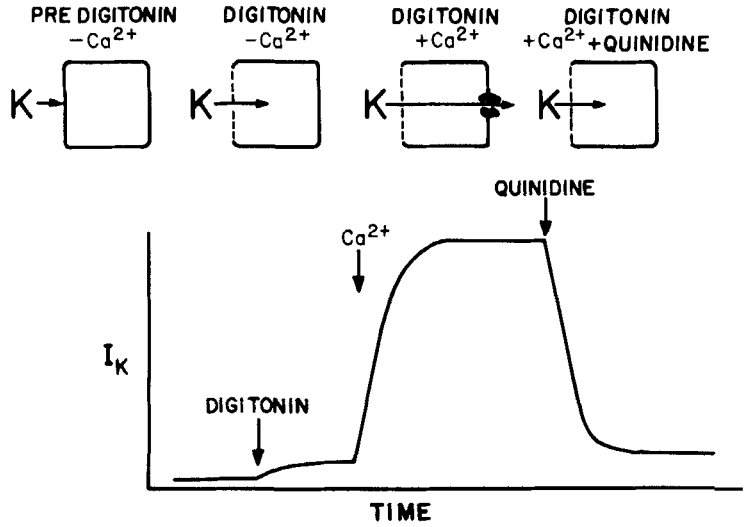

FIG. 9. The use of digitonin to permeabilize the apical membrane of turtle colon to monovalent cations and calcium. Transmural K gradient was imposed across isolated sheet of turtle colon mounted in an Ussing chamber. Mucosal solution was initially a potassium aspartate Ringer's, pH 6.6, buffered with EGTA so that the concentration of free calcium was ~1 n*M*. The serosal solution was sodium aspartate Ringer's containing 1 m*M* calcium and no EGTA. The addition of digitonin (20 μ*M*, mucosal) did not induce a transcellular K current, but the subsequent addition of sufficient calcium to raise the free concentration to $\sim 10^{-6}$ *M* induced a K current that was blocked by quinidine.

was blocked by quinidine. This conductance does not appear to be highly selective for K over Rb, however, and is probably not related to that activated by cell swelling.

D. Making Contact with K Channels: Fluctuation Analysis and Single-Channel Recording

Measurement of macroscopic ion currents across polyene- and digitonin-treated sheets of colonic epithelial cells has provided evidence for at least two types of ion channels which could be involved in K translocation across the basolateral membrane. We employed fluctuation analysis (Dawson *et al.*, 1985, 1986) and single-channel recording (Richards and Dawson, 1986a,b) in order to obtain more direct evidence for the existence of separate populations of channels, and we have utilized the fingerprints developed on the basis of macroscopic currents to attempt to identify putative channel populations.

1. Fluctuation Analysis of Basolateral K Currents

In studies conducted in the laboratory of Dr. S. I. Helman in collaboration with Dr. Helman and Dr. Willy Van Driessche, we measured the fluctuations in macroscopic K currents using polyene-treated turtle colon (Dawson *et al.*, 1985, 1986). Wills (1984) had previously studied basolateral K noise in nystatin-treated rabbit descending colon. Fluctuations were analyzed under conditions that corresponded to those described above for obtaining the two types of K conductance: under nonswelling conditions, with potassium gluconate Ringer's solution in the mucosal bath, and under swelling conditions with KCl Ringer's solution in the mucosal bath. Under nonswelling conditions, the power density spectrum (PDS) of the K current did not exhibit a Lorentzian component. The macroscopic current was unaffected by quinidine or lidocaine, and neither of these agents induced a Lorentzian component in the spectrum. Under swelling conditions (mucosal KCl), the currents were larger, as reported by Germann *et al.* (1986a,b), but PDS of the spontaneous noise did not consistently contain a Lorentzian component. The addition of 100 μM lidocaine to the bathing solutions, however, led to the appearance of a Lorentzian component in the PDS, with a corner frequency of about 60 Hz. Increasing the concentration of lidocaine in the bath increased the corner frequency of the PDS, as expected from a simple two-state model for reversible blockade. In experiments in which a K current was induced under nonswelling conditions and the addition of lidocaine did not induce a Lorentzian component in the PDS, changing the mucosal bath from potassium gluconate to KCl Ringer's solution in the presence of lidocaine induced the characteristic Lorentzian component in the PDS. This result is consistent with the notion that cell swelling is associated with the appearance of a new population of K channels which were not present in the normal resting membrane, but which are activated by the increase in cell volume. These experiments also illustrate a useful feature of blocker-induced fluctuations, the ability to track ion channels in a macroscopic setting using specific blockers.

It is of interest in relation to the patch–clamp results described below that 100 μM quinidine, which also blocks the swelling-induced K currents, did not induce a Lorentzian component in the PDS. This result is consistent with patch–clamp results (see below) which indicate different characteristics for block by this compound.

2. Single-Channel Recording

Richards and Dawson (1986a,b) applied the techniques described by Hamill *et al.* (1981) to cells from the turtle colon which were isolated by incubating mucosal scrapings in hyaluronidase. This treatment results in isolated cells that are rounded and do not retain any obvious structural indication of their polarity. We have identified at least two types of ion channels in these cells, one of which

appears to be highly selective for K. Frequently, in detached patches we encounter a channel that has a conductance of 30 pS and a zero reversal potential when bathed by identical concentrations of KCl and NaCl on either side. Although we have not characterized this channel in detail, its properties resemble those reported for calcium-activated, nonselective cation channels (Yellen, 1982).

Very frequently in on-cell patches, we encounter a channel that may have some direct relation to our macroscopic observations. This channel is often seen when the isolated cells are bathed by KCl Ringer's solution, a condition that is expected to promote cell swelling. In this condition we expect the membrane potential to be depolarized to around 0 mV. Accordingly, with NaCl Ringer's in the patch pipette ([K] = 2.5 m*M*), the reversal potential for this single-channel current is around 80 mV cell interior negative, and its conductance is from 17 to 20 pS. As shown in Fig. 10, the gating of the channel was sensitive to lidocaine. In the absence of the blocker, the channel was open much of the time, but exhibited "flickery" closings. This continual flicker is a gating characteristic by which we recognize this channel in recordings. The addition of 100 μ*M* lidocaine to the bath increased the flicker such that the channel was spending most of its time in a closed or blocked state. The effect of lidocaine was reversible and dose dependent. The effect of lidocaine on the behavior of single K channels was thus completely consistent with the result of the fluctuation analysis which showed a lidocaine-induced Lorentzian component under swelling conditions. Additional experiments (Richards and Dawson, 1986a) showed that both the swelling-induced conductance and the lidocaine-sensitive channels are highly selective for K over Rb.

Single-channel recordings also revealed that this K channel is blocked by both lidocaine and quinidine, but showed that the mechanism of block may be very different for the two molecules. As shown in Fig. 10, quinidine, rather than producing flicker, produced long closed periods punctuated by occasional short openings. In view of this result, it is perhaps not surprising that this concentration of quinidine did not induce a discernible corner frequency in the range of 10–100 Hz.

Taken together, these observations are consistent with the notion that the

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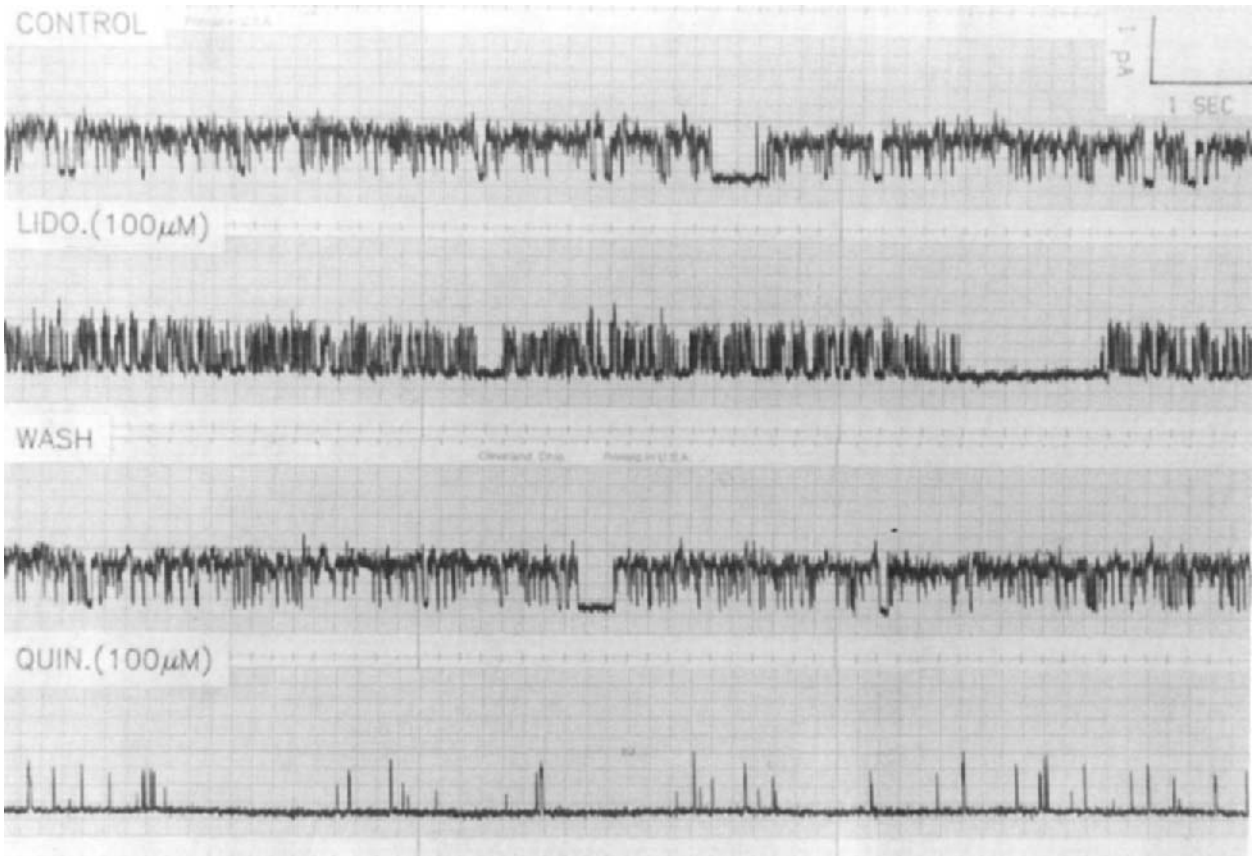
FIG. 10. Single-channel recordings from isolated colonic epithelial cells. Cells were isolated by incubating mucosal scrapings with hyaluronidase (1 mg/ml for 20 min) and patch clamped according to the methods described by Hamill *et al.* (1981) using Corning 7052 glass for patch pipettes (Rae and Levis, 1984). Top record is on-cell patch with NaCl Ringer's in the pipette and KCl Ringer's in the bath. Upward deflections represent outward K current at a holding potential of 0 mV. Under control conditions, channel was mostly open, but exhibited flickery closings. The addition of lidocaine (100 μ*M*) to the bath induced a high-frequency flicker and thus markedly reduced the mean current. The effect of lidocaine was fully reversible (wash) and the subsequent addition of quinidine (100 μ*M*) to the bath produced a dramatic block of the single-channel currents. (Data taken from unpublished record of Richards and Dawson.)


CONTROL
1 pA
1 SEC
LIDO.(100μM)
WASH
QUIN.(100μM)

resting and swelling-induced basolateral K conductances are due to two distinct populations of K channels one of which is characterized by a conductance of about 17–20 pS under physiological conditions and is specifically blocked by quinidine or lidocaine.

V. CONCLUSIONS AND DIRECTIONS

We are on the threshold of enormous increases in our understanding of the role of ion channels in epithelial transport processes. The application of single-channel techniques offers the opportunity to explore the diversity of channel types which may be involved in epithelial cell function. It seems reasonable to suspect that an understanding of the role of K channels in epithelial cells will require that we devise experimental strategies which will permit us to differentiate different populations of K channels that can be activated or inactivated under specific circumstances. In view of the diversity of K channels, it will be necessary to develop experimental systems where it is possible to keep track of different K channels at various levels of organization—from the cell layer to the isolated membrane patch—to permit the ion translocation process at the single-channel level to be related back to the transport function of the epithelium.

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Chapter 4

Role of Potassium in Cotransport Systems[1]

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I. INTRODUCTION

A role of potassium in cotransport, providing the driving force for the uptake of neutral amino acids into Ehrlich ascites tumor cells, had been proposed in 1958 by Riggs *et al.* A similar model was proposed by Eddy (1968) and by Eddy and Hogg (1969), who observed that a potassium gradient stimulates sodium-dependent neutral amino acid transport in the same cell. A contributory role of a potassium gradient has also been postulated by Crane for the sodium-D-glucose cotransport system (Crane, 1965). Subsequent studies of these systems suggested, however, that the effect of the potassium gradient was mainly due to the generation of a diffusion potential, which facilitated the electrogenic transfer via the sodium amino acid or sodium glucose cotransport system.

The introduction of membrane vesicles and of indicators for the membrane potential in intact cells made it possible to investigate cotransport processes under conditions in which the membrane potential is defined. Thereby an indirect role of potassium, generating a diffusion potential, could be distinguished from a direct role of potassium as cotransported cation.

In studies of this type, it has become apparent that there are two major ways in which potassium can affect sodium cotransport systems. One is the possibility that, due to the low specificity of the sodium binding site of the cotransport system, potassium can replace sodium at its binding site and thereby act as a substitute for sodium. This seems to be the case for the phenylalanine uptake in the gut of some butterfly species (Hanozet *et al.*, 1980) and in the brush border of

[1] Cotransport is used in the broader sense, which includes symport, antiport, and "cycloport."

the mouse small intestine (Berteloot *et al.*, 1982) "nonobligatory sodium cotransport systems." The other major role of potassium is that it is transported in addition to and concomitantly with sodium and the cotransportate. This cotransport of potassium can either be a symport, where potassium, sodium, and the cotransportate move in the same direction, or an antiport, where potassium moves in the opposite direction of sodium and the cotransportate or a combination of both processes.

A symport system has been discovered and studied in detail in the Ehrlich ascites tumor cells by Geck *et al.* (1980). It represents a sodium–potassium–chloride cotransport system. This system has now been found in a variety of cells and is involved in volume regulation or in transepithelial chloride transport (Geck and Heinz, 1986). An antiport system has been found for glutamate in synaptosomes (Kanner and Marva, 1982; Kanner and Sharon, 1978a,b) and for 5-hydroxytryptamine in platelets (Nelson and Rudnick, 1979).

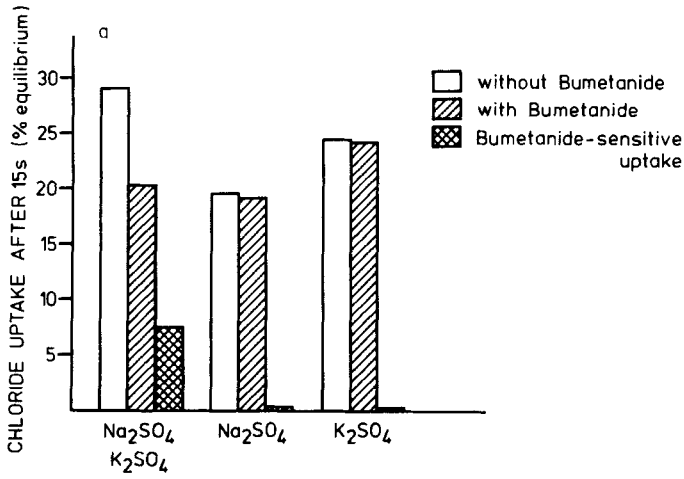
This chapter deals with the role of potassium in renal sodium cotransport systems. Symport, antiport, and "cycloport" systems will be described, with special emphasis on the demonstration of flux coupling between the cotransported solutes sodium, potassium, and cotransportate, and a model will be proposed which reconciles the opposing effects of potassium, such as symport and antiport, on the basis of differences in the cooperativity in their binding to the translocator.

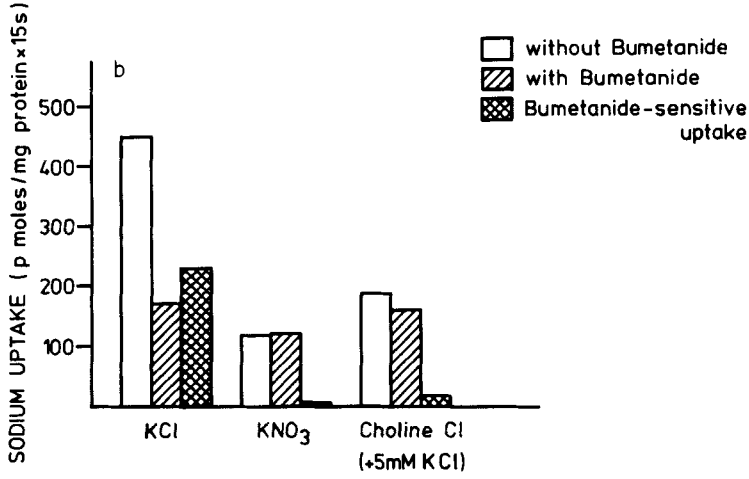
II. ROLE OF POTASSIUM IN RENAL SODIUM COTRANSPORT SYSTEMS

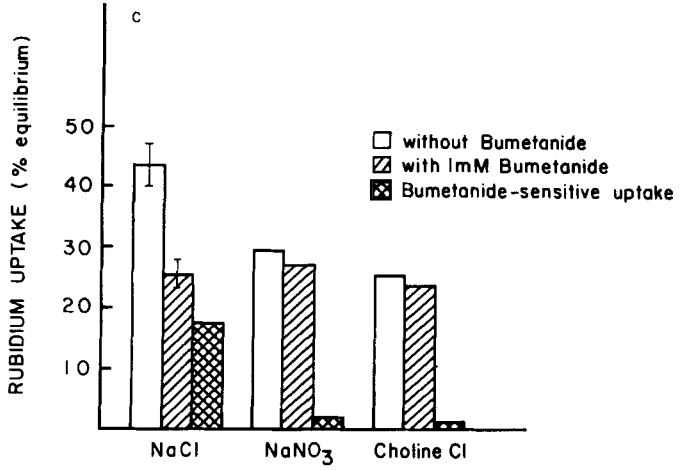
A. Symport

One of the best studied examples of a symport of potassium together with sodium and another solute is the NaCl–KCl cotransport system in the luminal membrane of the thick ascending limb of Henle's loop. First evidence for the presence of this cotransport system was derived from the observation that sodium chloride reabsorption in dissected microperfused segments of the thick ascending limb of rabbit kidney is dependent on the presence of potassium in the luminal perfusion fluid (Greger and Schlatter, 1981). The coupling between the sodium, chloride, and potassium flux could be studied directly in plasma membrane vesicles derived from isolated cells of the thick ascending limb (König *et al.*, 1983; Hannafin and Kinne, 1985). As shown in Fig. 1, these vesicles exhibit

FIG. 1. Symport of potassium: sodium chloride/potassium chloride cotransport. Flux coupling in plasma membranes prepared from isolated cells of rabbit kidney thick ascending limb of Henle's loop. Uptake of solutes was measured by a rapid filtration technique. (a) Chloride; (b) Sodium; (c) rubidium. [Values are compiled from König *et al.* (1983) and Hannafin *et al.* (1985). Reprinted with kind permission.]


a
CHLORIDE UPTAKE AFTER 15s (% equilibrium)
30
25
20
15
10
5
without Bumetanide
with Bumetanide
Bumetanide-sensitive uptake
Na_2SO_4
K_2SO_4
Na_2SO_4
K_2SO_4


b
SODIUM UPTAKE (p moles/mg protein x 15s)
500
400
300
200
100
without Bumetanide
with Bumetanide
Bumetanide-sensitive uptake
KCl
KNO_3
Choline Cl
(+5mM KCl)


c
RUBIDIUM UPTAKE (% equilibrium)
50
40
30
20
10
without Bumetanide
with 1mM Bumetanide
Bumetanide-sensitive uptake
NaCl
$NaNO_3$
Choline Cl

bumetanide-sensitive chloride, sodium, and rubidium fluxes. The bumetanide-sensitive chloride flux is dependent on the presence of both potassium and sodium; the bumetanide-sensitive sodium flux requires chloride and potassium, and sodium and chloride are necessary to demonstrate bumetanide-dependent rubidium flux. The stimulatory effect of potassium on sodium and chloride fluxes was also observed in equilibrium tracer exchange, where the effect of electrical potentials on the transport is eliminated. The latter finding and the demonstration of a bumetanide-inhibitable sodium- and chloride-dependent rubidium flux clearly indicate that potassium is cotransported together with sodium and chloride via a sodium chloride–potassium chloride cotransport system. Furthermore, binding studies using labeled "loop diuretics" such as bumetanide or *N*-methylfurosemide have demonstrated a direct interaction of the transport system with sodium, potassium, and chloride (Forbush and Palfrey, 1983; Hannafin *et al.*, 1983). Electrophysiological studies (Greger and Schlatter, 1983) as well as studies on membrane vesicles (Hannafin and Kinne, 1985) have shown that the transport system is electroneutral and has a stoichiometry of one sodium, one potassium, and two chloride. The electroneutrality, stoichiometry, and sensitivity to loop diuretics such as bumetanide are properties similar to those of the sodium chloride–potassium chloride system described in other epithelia as well as in Ehrlich ascites tumor cells, tissue culture cells, and avian erythrocytes (Geck and Heinz, 1986).

As compiled in Table I, the NaCl–KCl symport system has an apparent affinity for potassium of about 1 m*M* when investigated in membrane vesicles in the presence of 100 m*M* NaCl (Kinne *et al.*, 1985) and of 22 m*M* when studied in the presence of 0.5 m*M* sodium and 100 m*M* chloride (König *et al.*, 1983). Sodium thus exhibits a strong positive cooperativity with regard to potassium, facilitating its interaction with the transport system. With regard to the specificity of the potassium binding site, rubidium and ammonium can replace potassium to

TABLE I
INTERACTION OF POTASSIUM WITH RENAL SODIUM COTRANSPORT SYSTEMS

System	Specificity	Affinity (K_m)[a]
NaCl/KCl cotransport	$K \geq NH_4 \geq Rb >> Li, Na$	~1 m*M* (100 m*M* NaCl) 22 m*M* (0.5 m*M* Na, 100 m*M* Cl)
Sodium–glutamate cotransport	$K = Rb > Cs > NH_4 >> Li, Na$	~10 m*M*
Sodium–citrate cotransport	Not known	Not known

[a] Values in parentheses indicate the experimental conditions under which the affinity was determined.

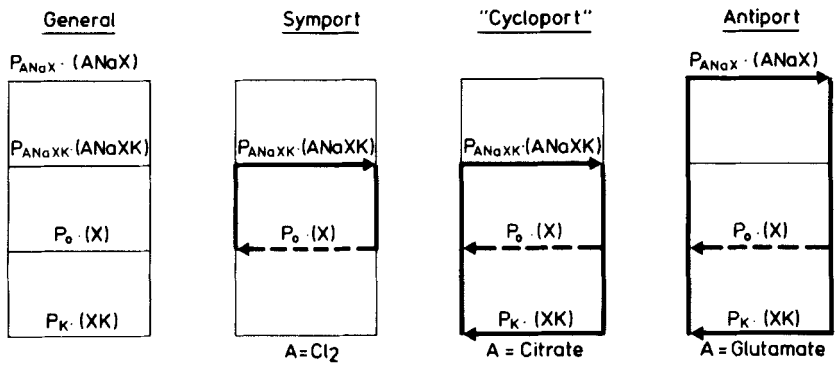


FIG. 2. General model for the effect of potassium on obligatory sodium cotransport systems: symport, antiport, and cycloport. Abbreviations: X, transporter; A, anion; Na, sodium; and K, potassium. The contribution of protons has been neglected for the sake of simplicity.

a similar extent, cesium is less effective, and sodium and lithium are not accepted (Kinne *et al.*, 1985).

A simple kinetic model for the cotransport of sodium, potassium, and chloride is depicted in the second panel of Fig. 2. The following steps are involved in the transport: binding of sodium, potassium, and chloride to the carrier, translocation of the carrier loaded with sodium, potassium, and chloride, dissocation of the ions from the carrier, and return of the unloaded carrier. The return of the latter is rate limiting for the overall transport rate.

B. Antiport

Antiport or exchange with potassium has been invoked in sodium–potassium glutamate cotransport across the luminal and contraluminal plasma membrane of the proximal tubule (Burckhardt *et al.*, 1980; Schneider and Sacktor, 1980; Sacktor *et al.*, 1981a,b; Sacktor, 1981). As shown in Fig. 3, a potassium gradient across the brush border membrane elicits different effects dependent on its orientation. If the gradient is directed into the vesicle, a slight inhibition of sodium-dependent uptake is observed. If directed from inside the vesicle to the outside, however, a marked stimulation of uptake is observed. The presence of an inside-out potassium gradient also leads to an intravesicular accumulation of glutamate. This result strongly suggests that the energy of the potassium gradient can by flux coupling be used to drive glutamate against its concentration gradient. The stimulation of sodium–glutamate cotransport by antiport of potassium can also be observed in efflux studies (Sacktor *et al.*, 1981a). Thus, in the presence of sodium and glutamate on the cis side, potassium is capable of reacting with the trans side of the transporter both at the outer and the inner

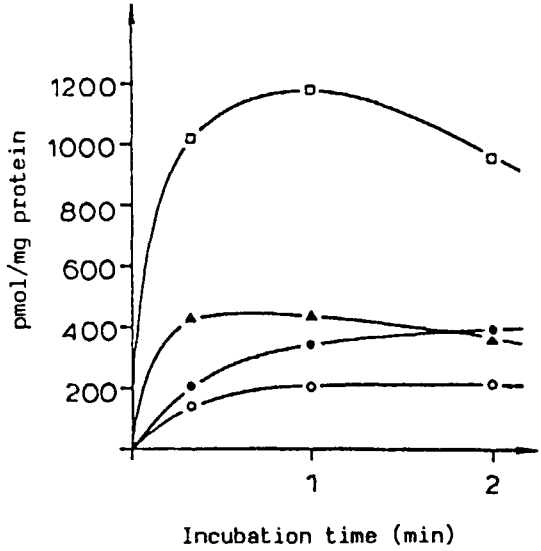


FIG. 3. Antiport of potassium: sodium–potassium–glutamate cotransport. Uptake of L-glutamate into brush border membrane vesicles isolated from rat kidney cortex was studied under sodium gradient conditions. The uptake was determined under four different conditions: (●), no K^+ present inside the vesicles ($[K_i^+] = [K_o^+] = 0$ m*M*); (▲), K^+-equilibrated ($[K_i] = [K_o] = 50$ m*M*); (○), K^+ gradient ($[K_i] = 0$ m*M*, $[K_o] = 50$ m*M*); (□), K^+ gradient ($[K_i] = 50$ m*M*, $[K_o] = 8.3$ m*M*). [Data are taken from Burckhardt *et al.* (1980) with kind permission.]

surface of the brush border membrane. These results suggest that coupling of glutamate and potassium flux via antiport occurs to the extent that electrical driving forces can be excluded. Since the stimulation by potassium is also observed in the presence of permeant anions, a merely electrical coupling seems unlikely, even though in the rat the translocation of sodium glutamate is electrogenic positive (Burckhardt *et al.*, 1980). Direct coupling between L-glutamate flux and potassium flux has been demonstrated by Koepsell *et al.* (1984) in proteoliposomes reconstituted from renal brush border membranes. In this preparation an L-glutamate gradient, directed from inside of the liposome to the outside, stimulated rubidium uptake in the presence of sodium.

As depicted in Table I, potassium has an apparent affinity of ~10 m*M* to the sodium–glutamate cotransport system in rat kidney and of 172 m*M* in rabbit kidney. Potassium can be replaced with equal potency by cesium, to a lesser extent by ammonium, but not by lithium or sodium (Burckhardt *et al.*, 1980; Turner, unpublished results).

It should be noted that there is some controversy in the literature about the rheogenicity of the sodium–glutamate symport–potassium antiport system. In

the rat, the system seems to be electrogenic positive (Burckhardt *et al.*, 1980), whereas in the rabbit the transport seems to be electroneutral (Schneider and Sacktor, 1980). Part of the cations translocated with glutamate represent protons, as demonstrated by Nelson *et al.* (1983). Thus, an inward proton gradient in the presence of sodium and potassium can accumulate glutamate inside rabbit brush border vesicles. Potassium and protons seem to compete for the cation binding site located at the inner surface of the transport system, which is involved in the antiport pathway (Nelson *et al.*, 1983).

With regard to the stoichiometry of the transport, recent studies by Turner *et al.* indicate that one glutamate ion is symported with two sodium ions and antiported with one potassium ion (Turner, unpublished results). A kinetic model for the glutamate transport is depicted in the fourth panel of Fig. 2. It includes the binding of sodium and glutamate to the translocator at the outside of the membrane, translocation of the complex, dissociation at the inner surface of the membrane, and return of the carrier loaded either with potassium or in the empty form. The translocation of the latter is assumed to be the slowest. For the sake of simplicity, protons, even though they presumably participate in this cotransport, are not included in this model.

C. Cycloport

In the sodium citrate cotransport system, in contrast to the transport systems described so far, potassium exhibits its effect most strikingly when present in equal concentrations at both sides of the membrane (Grassl *et al.*, 1983). As shown in Fig. 4, in the presence of sodium, potassium significantly stimulates tracer exchange as well as Na gradient-driven net transport of citrate in the vesicles. On the other hand, potassium gradients in either direction did not affect citrate transport markedly. The observation that K^+ stimulates citrate fluxes only in the absence of a K^+ gradient could be explained by the hypothesis presented in Fig. 2 that potassium interacts both with the loaded and the unloaded carrier, thereby facilitating simultaneously both the forward step and the backward step of the transport cycle. Thus, symport and antiport of K seem to be involved in this transport, a phenomenon which we would like to call cycloport.

As in glutamate cotransport, protons also affect citrate transport (Wright *et al.*, 1982; Grassl *et al.*, 1983). This effect has been neglected in the current considerations.

A hypothetical model for the transport of citrate in the presence of potassium is depicted in Fig. 2 in the third panel. The scheme includes binding of sodium, citrate, and potassium at the outer surface of the membrane, translocation, dissociation of sodium and citrate from the carrier at the inner surface of the membrane, and a recycling of potassium by return of the carrier with potassium.

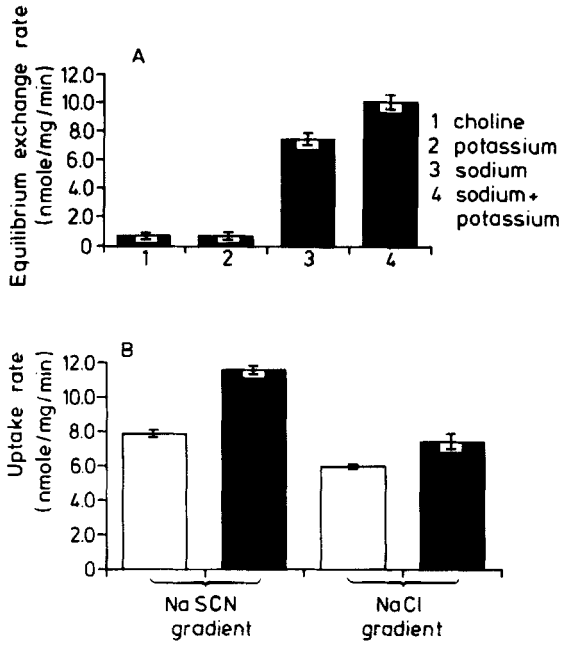


FIG. 4. Cycloport of potassium: sodium–potassium–citrate cotransport. Uptake of L-citrate into brush border membrane vesicles isolated from calf kidney. (A) Tracer exchange; (B) net uptake; (□), no K; ■, $K_i = K_o = 100$ m*M*. [Data are taken from Grassl *et al.* (1983) with kind permission.]

The rate of translocation of the empty carrier is probably the lowest and may therefore, in the absence of K^+, limit the overall transport.

III. THEORETICAL CONSIDERATIONS

In Fig. 2, the three different transport schemes involving potassium are compiled. They can be incorporated into a general scheme in which, for the different transport systems only, some of the possible pathways are postulated to contribute to the overall transport.

Now the question arises why in the various systems only certain pathways are used; e.g., why in the NaCl/KCl cotransport system K is symported and why in the glutamate cotransport system K is antiported. One way to explain this phenomenon is to consider the cooperativity between the different ligands, which can determine to what extent a certain carrier–ligand complex exists and, thus, whether a certain transport pathway participates in the transport cycle.

Cooperativity, for example, between sodium and potassium can be described by r_{KNa}, which is defined as

$$r_{KNa} = \frac{K_{Na}}{K_{KNa}} = \frac{K_K}{K_{NaK}} \tag{1}$$

If the affinity of the carrier for K after interacting with sodium is higher or the apparent dissociation constant K smaller, r_{KNa} becomes larger than unity, which is defined as positive cooperativity. For thermodynamic reasons, the same holds for the increase in sodium affinity after the formation of the K–carrier complex.

Negative cooperativity (r_{KNa} smaller than unity) would be observed if the presence of sodium decreases the affinity of the carrier for potassium, and vice versa. The two other cooperativity factors that will be considered are r_{NaA}, which describes the cooperativity between sodium and the cotransportate, and r_{KA}, which describes the cooperativity between potassium and the cotransportate (Table II). For the binding of any ligand to a given species of the carrier, the overall cooperativity, i.e., the product of all r values, is decisive.

With regard to r_{KNa}, it has been observed in our studies as well as in the studies of Rindler *et al.* (1982) and of Saier and Boyden (1984) that there is a strong positive cooperativity between Na^+ and K^+. K^+ increases the affinity of the system for sodium, and vice versa. This positive cooperativity we assume makes symport of K with sodium and chloride possible.

Negative cooperativity between K and Na, on the other hand, could explain the antiport effect of K in the glutamate cotransport system. Glutamate uptake into the brush border in the presence of sodium is not stimulated by potassium, but (slightly) inhibited. Antiport occurs only with potassium and is reduced when

TABLE II
COOPERATIVITY IN OBLIGATORY SODIUM COTRANSPORT SYSTEMS[a,b]

Coefficient of cooperativity	Symport	Antiport
$r_{NaA}\left(=\frac{K_A}{K_{Na-A}}=\frac{K_{Na}}{K_{A-Na}}\right)$	>1	>1
$r_{KA}\left(=\frac{K_A}{K_{K-A}}=\frac{K_K}{K_{A-K}}\right)$	>1	<1
$r_{KNa}\left(=\frac{K_{Na}}{K_{K-Na}}=\frac{K_K}{K_{Na-K}}\right)$	>1	<1

[a] Effects of any interfering third ligand are neglected.

[b] Among nonobligatory systems are the transport systems in the butterfly gut and mouse small intestinal brush border mentioned above.

sodium is present simultaneously with potassium at the trans side. The weakened interaction between potassium and the carrier in the presence of sodium, and vice versa, is consistent with the view that the cooperativity between sodium and potassium is negative ($r_{KNa} < 1$). The negative cooperativity would not favor symport between potassium and sodium (and glutamate), but an antiport with potassium alone.

In all sodium cotransport systems studied so far, positive cooperativity between sodium and the cotransportate ($r_{NaA} > 1$) has been observed. This includes the Na–K–Cl symport system, the glutamate transport system, and the citrate transport system.

With regard to r_{KA}, a positive cooperativity has been found between K^+ and Cl^- in the Na–K–Cl cotransport, but the corresponding cooperativity between K and glutamate in the glutamate cotransport has to be assumed to be negative ($r_{KA} < 1$). If r_{KA} were greater than unity, in the presence of sodium a stimulation of glutamate uptake by extravesicular K^+ and stimulation of glutamate efflux by intravesicular K^+ would be expected. A negative cooperativity between K and glutamate also explains that in the absence of Na^+, K^+ is not cotransported with glutamate. Thus, a change of both cooperativity factors r_{KNa} and r_{KA} from a value of larger than unity to a value smaller than unity can explain the difference between a potassium symport system such as the Na–K–Cl cotransport system and a potassium antiport system such as the glutamate cotransport system.

The different cooperativities postulated for the two transport systems just discussed are summarized in Table II.

The sodium citrate cotransport system represents another interesting case, which can be considered as a combination of symport and antiport (Grassl *et al.*, 1983), as potassium seems to bind to the carrier both in the presence or absence of sodium. In contrast to the previously discussed systems, however, the effects of K^+ observed with citrate transport can hardly be accounted for by the cooperativity effects alone, as will be discussed in conjunction with the consideration of velocity effects.

So far, we have not considered whether and to what extent velocity effects have to be invoked to explain the actions of K on the various cotransport systems. The above symport and antiport systems could, at least qualitatively, be sufficiently explained by affinity effects alone, namely, on the basis of cooperativity between the ligands involved. This does not exclude, of course, that velocity effects are involved as well. Obviously, the effects of K^+ on these systems could also be formally explained by velocity effects alone, i.e., without invoking any cooperativity at all (Heinz *et al.*, 1972). But this would require certain implausible, even farfetched assumptions. For instance, in symport with Na, we would have to postulate that binding of this cation inhibits translocation

of the unloaded carrier, but accelerates translocation of the carrier loaded with the transportate. On the other hand, to explain antiport with K analogously, we would have to postulate exactly the reverse behavior for K^+: that it accelerates translocation of the unloaded carrier and inhibits translocation of the loaded carrier. It is difficult to reconcile these opposing effects of the two cations within a plausible and unifying model based on velocity effects only. Hence, we are inclinded to attribute to the cooperativity effects a dominating, though not necessarily exclusive role in most of these systems.

There is one system, though, which clearly seems to demonstrate that velocity effects are not only involved, but even essential to account fully for all effects observed, namely, citrate transport (cycloport). Here, an overall positive cooperativity between K and the other ligands, Na and citrate, would account for the observed stimulation of both equilibrium exchange and Na gradient-driven net transport of citrate by equal K on both sides, but not for the observed absence of symport between K and citrate. This can hardly be explained otherwise than by a true velocity effect of K in that its binding to the carrier appreciably facilitates the translocation of at least the unloaded carrier, but most likely also that of the fully loaded one (Grassl *et al.*, 1983). Hence, velocity effects of K^+ are not only necessary, but also sufficient to explain qualitatively all observations with this cation in citrate transport.

We therefore believe that in all systems of secondary active transport, both affinity and velocity effects are simultaneously involved in the action of K^+, but that in some systems the affinity effect dominates, and in others the velocity effect dominates.

Unfortunately, we cannot as yet offer a plausible and unifying model for such velocity effects, quite in contrast to the cooperativity effects, to which the well-known allosteric enzyme models such as that of Monod *et al.* (1965), that of Koshland *et al.* (1966), and others could be applied. The question arises whether, for instance, each ligand imparts its own specific velocity effect on the carrier species to which it attaches and whether the resulting overall velocity of the fully loaded carrier is a definable function of the individual contributions. Or it could be that the carrier can exist in two conformations only, a mobile one and an immobile one, and that, in analogy to allosteric systems, the distribution of the two forms depends on the ligands attached. If so, how would such a conformational state relate to the postulated affinity effects? Before some plausible models along these lines are available, the question regarding the molecular basis of the postulated affinity and velocity effects must be left open.

Finally, it should be noted that for the sake of simplicity, the effects of protons on the glutamate transport and the citrate transport have not been included in our discussion. The aim of this chapter was to discuss the effect of potassium; the role of protons in these systems remains to be elucidated.

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Chapter 5

Functional Roles of Intracellular Potassium in Red Blood Cells

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I. INTRODUCTION

While the primary focus of this chapter is on various specialized functions of cellular K (K_i), it should be recognized that this particular bias results in the present instance not only from our participation in a symposium honoring Robert W. Berliner for his contributions to K metabolism, but also from our desire to highlight areas of mutual interest from a different perspective. We will, therefore, consider certain red cell cation transporting systems, their characteristics, evident control mechanisms, and cellular roles.

II. GENERAL COMMENTS

Living cells are known to contain K_i in high and Na_i in low concentrations, whether they are bathed by pond water or by solutions high in Na_o and low in K_o, such as plasma and seawater. These gradients of Na and K that characteristically exist across cell membranes reflect not only the input of metabolic energy, but also the operation of specific transport mechanisms responsible for their maintenance and perhaps their initial establishment. In addition, the ionic components of contemporary cellular and extracellular environments in mirroring evolutionary history may also echo the composition and abundance of elements in the primordial past (cf. Steinbach, 1962).

Thus, the adaptation to high K_i is reflected in a variety of cellular processes such as the dependence of enzyme activities and metabolism on high K_i (Suelter,

1970), the requirement of K_i for protein synthesis [translation requires K_i (e.g., Burns and Rozengurt, 1984)] in growth and differentiation, the physiological roles ion gradients provide in excitatory processes (as in nerve and muscle) and in solute movements by cotransport and by countertransport mechanisms.

The physical state of intracellular ions, such as Na_i, K_i, and Cl_i, is important to know in order to define and characterize their membrane gradients and the forces that operate on their movements. While information of this kind is not available for many cell types (cf. Edzes and Berendsen, 1975; Tsien, 1983) and is evidently not easily attainable, it seems clear, at least for mammalian red cells, that the thermodynamic activity Na_i, K_i, and Cl_i have in the cell interior is the same as the activity each of these ions has in free solution (Freedman and Hoffman, 1979). In addition, measurements (Williams *et al.*, 1959) indicate that red cells are in osmotic equilibrium with their environment (because the activity of water on the two sides of the membrane is the same) and that all of the cellular water is available for the solution of ions such as K_i and Cl_i (Cook, 1967). Another indication that there is no significant binding or compartmentation of Na_i, K_i, or Cl_i in red cells is the fact that by using the concentrations these ions have on the two sides of the membrane, the membrane potential, perturbed in a variety of ways, adheres to and can be described by the constant field equation (cf. Hoffman and Laris, 1974; Rink and Hladky, 1982).

III. RED CELL VOLUME AND THE Na/K PUMP

The operation of ion transport mechanisms provides the basic way cells have for steady-state maintenance of their cell volume and for their homeostatic adjustments to environmental perturbations. Cell volume, of course, circumscribes body size and for red cells is critical in defining their flow properties through the microvasculature. [Because of their size, red cells must deform in order to pass through the capillary bed and their deformability is decreased in shrunken and swollen states (see Clark *et al.*, 1983).] Red cells evidently regulate their cell water, and hence their cell volume, by controlling the intracellular sum of Na + K. A pump/leak hypothesis has been proposed as the principal mechanism responsible for maintaining this sum constant (Tosteson and Hoffman, 1960). In this model, the active transport of both Na and K moving uphill act to precisely counterbalance the passive losses that occur by these ions moving down their respective electrochemical gradients. Quantitative tests of this model in high K_i and low K_i type sheep red cells, whose relative cell volumes are essentially the same, appear to account for the differences in the steady-state composition of Na_i and K_i by differences in pump rates and leak permeabilities.

While the pump/leak model appears to be an adequate mechanism describing the maintenance of normal cell volume, this is not the case in many types of red cells, e.g., bird red cells, when their volume is altered by various means. In these

instances (recently reviewed by Siebens, 1985), changes in cell volume transiently activate regulatory cotransport and countertransport mechanisms that promote net movements of Na and K leading to readjustments in cell content and the return to a steady-state cell volume.

It may not be evident in the foregoing discussion that the metabolically dependent reciprocal interplay between Na_i and K_i is a primary reflection of Na/K pump function. Properties of the human red cell Na/K pump that are important in these and subsequent considerations are that the influx of K is tightly coupled to the efflux of Na such that 3 Na_i are exchanged for 2 K_o (Post and Jolly, 1957) at the expense of one ATP (Sen and Post, 1964). The difference in 3 Na_i/2 K_o stoichiometry is the basis for the pump being electrogenic, moving net charge (Na) outward (Hoffman *et al.*, 1979). In normal cells, the membrane potential is about −9 mV (equal to the Donnan equilibrium potential for Cl at 37°C, pH 7.4) and the pump's contribution to it is presumably negligible, being less than −0.2 mV (Hoffman and Laris, 1984). The average red cell contains 200 to 300 pumps, each transporting about 150 ions/site · sec (Hoffman, 1969; Ingram, 1970; Joiner and Lauf, 1978a). The concentration of Na_i (in the presence of K_i) giving half-saturation of Na efflux is ~ 15–20 m*M*, while the concentration of K_o giving half-saturation of K influx (in the presence of Na_o) is 1–2 m*M* (see Cavieres, 1977). There is evidence that the number of pumps per cell in different normal individuals varies inversely with the concentration of Na_i, inferring that no such variation occurs in the leakage permeabilities (Ingram, 1970). [Whether this is related to changes in red cell Na_i and transport parameters reported for hypokalemic humans and rats is not at present known (Chan and Sanslone, 1969; Rubython and Morgan, 1983).] The Na/K pump is comprised of two types of subunits (Kyte, 1971), each of which has now been cloned (Kawakami *et al.*, 1985; Shull *et al.*, 1985; Noguchi *et al.*, 1986). The α subunit (lamb kidney) contains 1016 amino acid residues and has a calculated M_r of 112,177; the β subunit (torpedo electroplax) is glycosylated, contains 305 amino acid residues, and has a calculated M_r of 34,671. While α/β stoichiometry is known to be 1 to 1, it is not yet clear whether translocation of Na and K involves monomeric (αβ) or dimeric ($\alpha_2\beta_2$) forms of the subunits (see Glynn and Ellory, 1985). ATP interacts with and phosphorylates the α subunit on its inward-facing aspect (Uesugi *et al.*, 1971), while cardiotonic steriods (e.g., ouabain), known to be highly specific inhibitors of the Na/K pump, bind to an outward-facing portion of the α subunit (Ruoho and Kyte, 1974; Forbush *et al.*, 1978) at a site that is antagonized by K_o (Glynn, 1957; Hoffman, 1966).

IV. COMPETITIVE EFFECTS OF K

We now consider certain types of interactions between Na and K on the same side of the membrane that result in alterations in the Na/K pump rate. These

interactions take place on the outside as well as on the inside of red cell membranes. One type of interaction is that the apparent affinity of the pump for K_o can be increased by decreasing Na_o (Na_o being replaced, for instance, by choline) without any change in V_{max} (Post *et al.*, 1960). On the other hand, an analogous type of interaction occurs on the inside of the membrane (Hoffman, 1962; Knight and Welt, 1974; Garay and Garrahan, 1973) where the apparent affinity of the pump for Na_i is increased by lowering K_i, again without any appreciable change in V_{max}. Because the pump operates below saturation at normal values of Na_i and K_o (about one-third to one-half V_{max}), these types of interactions function to modulate the pump rate beyond the changes in rate that would be expected to occur when Na_i or K_o are varied without any effects of K_i and Na_o, respectively. Thus, while the pump would be inhibited by decreasing Na_i, the inhibition would be greater because of the effect of K_i. Similarly, inhibition of the pump by decreasing K_o is increased because of the interaction with Na_o. An important consequence of this kind of alteration by K_i and Na_o of the apparent affinities of the pump for Na_i and K_o is that, in terms of the pump/leak model, the steady-state volumes assumed by the cells would be contingent on the extent of the modulatory effects. (For the situation described, the set for cell volume would increase as the apparent affinities are decreased.) It should also be noted that while Na and K interact with each other presumably on the same form of the pump intermediate (recognizing that the intermediate form involved on the inside may be different from the one on the outside), it is not always clear whether they interact competitively at the same sites or allosterically at separate sites. It is also of interest to note that the sigmoidicity of K_o activation of K influx through the pump as influenced by Na_o (Post *et al.*, 1960) may be dependent upon the presence of K_i (cf. Karlish and Stein, 1985).

The above forms of interactions between Na_i and K_i are also seen when the pump is carrying out Na/Na and K/K exchange (see Sachs, 1986). Thus, K_i acts to inhibit Na/Na exchange by decreasing the apparent affinity of the pump for Na_i and, alternatively, Na_i can act to inhibit K/K exchange by decreasing the pump's apparent affinity for K_i. While in each instance the form of the interaction between Na_i and K_i is evidently competitive, Na/Na and K/K exchanges are associated with different intermediates in the reaction cycle of the pump. It has yet to be determined whether the binding sites involved are the same in the different transport modes.

Another kind of interaction of K_i with the Na/K pump occurs in low K sheep red cells where it has been demonstrated that in normal circumstances, the Na/K pump is inhibited by K_i (Hoffman and Tosteson, 1969). Exposure of the cells to a specific antibody relieves the inhibition and stimulates the Na/K pump by several fold (Lauf *et al.*, 1970). This action of K_i has been found to be noncompetitive (Dunham and Anderson, 1987). In addition, it was also shown that alteration in K_i could affect the binding of the antibody to the cell (Farquharson and Dunham, 1986).

V. EFFECTS OF K_i AND Na_i ON OUABAIN BINDING

We have previously shown, in resealed human red cell ghosts, that inside ATP promotes the binding of ouabain to the membrane with concomitant inhibition of the Na/K pump (Bodemann and Hoffman, 1976). The ouabain binding rate is also markedly influenced by K_i and Na_i provided that K_o is also present. Thus, when K_i is low (e.g., 4 m*M*), increasing Na_i *decreases* the rate of ouabain binding (Bodemann and Hoffman, 1976), in contrast to the situation when K_i is high (e.g., 80 m*M*), where increasing Na_i *increases* the ouabain binding rate (Joiner and Lauf, 1978b). It turns out that when K_i is held constant, at about 40 m*M*, the ouabain binding rate is insensitive to changes in Na_i (Bodemann *et al.*, 1983). Furthermore, a similar set of effects can also be observed when K_i is varied at different set values of Na_i. In this instance, it was found that when Na_i was held constant, at about 30 m*M*, the ouabain binding rate was insenstive to changes in K_i (Bodemann *et al.*, 1983). We have not been able to model these and other (e.g., when Na_i and K_i are varied reciprocally for different total values of the sum, $Na_i + K_i$) effects of Na_i and K_i on the ouabain binding rate with a single ion binding site of varying selectivity of Na versus K (M. Milanick and J. F. Hoffman, unpublished observations). Rather, separate Na and K binding sites appear to be minimally required together with assignment of the relative affinities of the various forms of the pump (E) that bind ouabain (i.e., E, NaE, EK, NaEK). This model is able to be fitted to a variety of results as well as satisfactorily predicting the null concentrations of K_i and Na_i that make the ouabain binding rate insenstitive to variations in Na_i in the former and K_i in the latter instance. However, while the relationship of the sites involved with Na_i and K_i modulation of ouabain binding with the sites involved with Na_i and K_i interactions on the various pump transport modes has yet to be established, there is no reason to think that they are different.

VI. K_i AND Na_i EFFECTS AND ATP/ADP RATIOS

We now turn to the effects of K_i and Na_o on the Na/K pump in resealed human red cell ghosts, made to contain high K_i and low Na_i and an ATP regenerating system in order to vary the concentration of ADP at set levels of ATP (Kennedy *et al.*, 1986). Na/K pump activity was assessed by measuring ouabain-sensitive Na efflux in a medium containing high Na_o and a saturating concentration of K_o. It was found, as expected, that Na/K exchange was inhibited by increasing concentrations of ADP. But the most dramatic result was that this inhibition of the pump could be relieved by the removal either of K_i (Marín and Hoffman, 1985) or of Na_o (Kennedy *et al.*, 1986) from their respective phases. [These results, by the way, can be mimicked by substitution of orthophosphate for ADP, but orthophosphate has no additional effects when high ADP is present (Marín

and Hoffman, 1985).] The pump's stoichiometry of 3 Na_i/2 K_o/1 ATP remained constant independent of variations in pump rate brought about by changes in ADP, K_i, or Na_o. We thought at first that these effects of K_i and Na_o removal reflected changes in the electrochemical potential gradients of these ions across the membrane, making it easier for the pump to transport ions with rather than against their respective concentration gradients. But when systematic variations in the gradients were studied, it was found that these effects were sided dependent only on the relative concentrations of K_i or Na_o (R. Marín and J. F. Hoffman, unpublished observations). Thus, these effects are kinetic rather than thermodynamic and may underlie the insensitivity of the pump fluxes of Na and K to changes in the membrane potential (Milanick and Hoffman, 1985). On the other hand, it might appear that these sided effects of K_i and Na_o (as well as ADP and orthophosphate) are simple, but that would be deceptive, since their explanation would appear to rest on considerations of the various intermediates involved in the pump's transphosphorylation cycle. While extensive analysis and discussion along this line is beyond our present scope, it is of interest to point out that Na_o and K_i must presumably be bound to regulatory sites in order to exert their effects, since these ions are not transported by the pump in these situations.

Another aspect of pump-associated regulatory sites has emerged from studies on a mode of the red cell Na/K pump referred to as uncoupled Na efflux (Garrahan and Glynn, 1967). Uncoupled Na efflux is an ATP-dependent, ouabain-sensitive efflux of Na that takes place in the absence of both Na_o and K_o. Addition of a low (e.g., 5 m*M*) concentration of Na_o inhibits uncoupled Na efflux, while higher concentrations of Na_o activate Na/Na exchange. Addition of K_o, of course, converts uncoupled Na efflux into Na/K exchange. A curious feature about uncoupled Na efflux is that not only was the process found to be electroneutral, but analysis showed that in place of Na_i exchanging for an external cation, it was coupled to the efflux of an intracellular anion (Dissing and Hoffman, 1983). In studies where chloride had been substituted by SO_4 on both sides of the membrane (and the cells DIDS treated to eliminate anion exchange by band 3), it was found that the Na_o-sensitive efflux of Na was twice the Na_o-sensitive efflux of SO_4, thus accounting for the electroneutrality of the process. Uncoupled Na efflux can be inhibited by using impermeant anions, such as tartrate, in place of SO_4. Neither Na/K exchange nor its electrogenicity is affected by impermeant anion substitution, indicating that an anion is not involved in this flux mode.

It is important for the present discussion to emphasize the roles Na_o and K_o have in modifying the transport reaction form of the pump as just described. Presumably Na_o, at high concentrations, binds to the pump, converting uncoupled Na efflux to Na/Na exchange with concomitant inhibition of anion efflux, while K_o binds to the pump, inhibiting anion efflux as well as converting the transport form to Na/K exchange. These actions of Na_o and K_o are more than

modulatory, since they act to define distinct transport modes of the pump. Thus, not only is the internal charge structure of the pump altered by the binding of Na_o and of K_o to the pump, but these interactions presumably occur prior to the release of Na and its accompanying anion in order to signal the pump that its reaction mode is changed (see also Beaugé and Berberian, 1983). It will be of interest to know the molecular basis for this kind of signaling and whether more than simple occupancy of the regulatory sites is involved, such as α–β subunit interaction. Considerations of this kind also raise the possibility that the sites for Na_o which have been proposed (Cavieres and Ellory, 1975; Sachs, 1977; Hobbs and Dunham, 1978) to act allosterically on other pump transport parameters may be the same as the regulatory sites discussed above.

By way of summary, it should be evident from the above survey that there appear to be at least two and perhaps four types of modulatory and/or reaction signal sites present on the Na/K pump complex. These sites are sided such that Na_i and K_i sites exist on the inside together with Na_o and K_o sites on the outside. While presumably the K_i- and Na_o-type sites can interact allosterically with the pump to alter transport, there is no evidence to indicate that Na_i and K_o interact at sites separate from those involved in their translocation. In addition, the relative affinities of these ions for their respective regulatory sites also remain to be determined.

ACKNOWLEDGMENT

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Part II

Renal and Extrarenal Control of Potassium: Physiology

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Chapter 6

Overview: Renal Potassium Transport along the Nephron

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I. INTRODUCTION

Berliner and Kennedy (1948) and Mudge *et al.* (1948) reported that mammalian kidneys regularly responded to certain experimental maneuvers by excreting potassium at rates exceeding the rate of potassium filtration. The phenomenon of potassium secretion and its contribution to renal potassium excretion continued to be an object of study by Berliner and his colleagues for the next decade and by his students and scientific offspring for what might be called the next generation.

In their paper, "Renal Mechanisms for Excretion of Potassium," Berliner *et al.* (1950) presented evidence indicating that potassium excretion depends on secretion of potassium at a distal tubule site and that the secretory process behaves as though it involves an exchange of reabsorbed sodium for secreted potassium. These conclusions were based on experiments that employed clearance methods and compared effects of intravenous infusions of solutions containing potassium along with sodium and several different anions.

Subsequently, Davidson *et al.* (1958) used another experimental approach to gain insight into the mechanism of potassium secretion. These experiments were designed to determine whether potassium excretion could be dissociated from the rate of potassium filtration. The glomerular filtration rate (GFR) of one kidney was reduced by constricting one renal artery. Separate measurements of sodium and potassium excretion by the two kidneys showed that when GFR was reduced

by about 30%, sodium excretion decreased by 80%, and potassium excretion decreased by 50%. In contrast, if the rate of sodium excretion was maintained at a somewhat higher level by infusing sodium salts and administering diuretics, then similar reductions in GFR did not decrease potassium excretion. This clear dissociation of potassium filtration and potassium excretion was taken as evidence that rates of potassium reabsorption and secretion are variable and independent, that the rate of potassium secretion is related to the rate of potassium intake, and that the site of potassium secretion is located distal to the site of potassium reabsorption. Summarizing these and other results in his lecture to the Harvey Society, Berliner (1961) stated that distal potassium secretion is the major determinant of potassium excretion. Graciously awarding credit for the idea to Alfred Gilman, he said that Gilman was the first person in the late 1940s to venture the bold suggestion that all the filtered potassium might be reabsorbed with the excreted potassium being derived totally from the secretory process.

Additional details that further explain and qualify these pioneering conclusions have been uncovered in the years since 1960. This review will briefly summarize observations that provide the basis for our current understanding of renal potassium handling.

II. ANATOMY

Some of the additional details arise from anatomical considerations. It is now known that more than just proximal reabsorption and distal secretion are involved. The schematic representation of a kidney in Fig. 1 shows subsegments along a superficial and a juxtamedullary nephron. The arrows along the way indicate the known (or probable) directions of potassium transport by most of the subsegments. Potassium is reabsorbed from the first and second segments of the proximal convoluted and straight tubules, but, as discussed by Jamison and Müller-Suur (this volume, Chapter 7), potassium is secreted into the third proximal segment, or into the descending thin limb, or into both. Potassium is then reabsorbed from the thick ascending limb, or the distal straight tubule as it is known by anatomical purists. A question of current interest is whether and under what circumstances these successive transport steps in the loop of Henle contribute to adaptive changes in final potassium excretion. Potassium is secreted into distal segments beyond the macula densa region. Three segments are distinguished in this figure: the dital convoluted tubule, the connecting tubule, and the cortical collecting duct. The major sites of potassium secretion seem to be the connecting tubule and the cortical collecting duct. Not all of this secreted potassium completes the journey to the final urine; the collecting duct passing through the inner stripe of the outer medulla appears to be a site of potassium

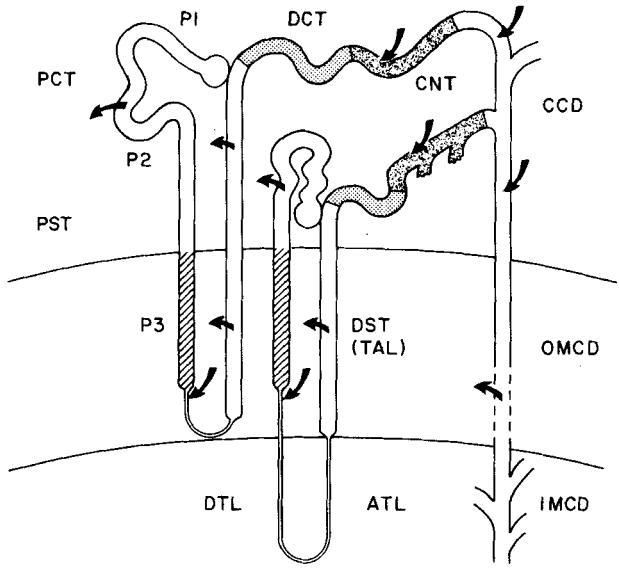

FIG. 1. Schematic representation of the principal segments of representative superficial (surface) and deep (juxtamedullary) nephrons. Arrows indicate direction of potassium transport (reabsorption or secretion). Nephron segments are identified as follows: PCT, proximal convoluted tubule; PST, proximal straight tubule; P1, P2, P3, first, second, and third segments respectively, of proximal tubule (P3 is hatched); DTL, ATL, descending and ascending thin limbs, respectively; DST, distal straight tubule or thick ascending limb (TAL); DCT, distal convoluted tubule (stippled); CNT, connecting tubule (shaded); CCD, cortical collecting duct; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct. [Reproduced from Wright and Giebisch (1985), Fig. 2).]

reabsorption. Studies of the inner medullary collecting duct have shown both net reabsorption and net secretion.

I will focus on the cortical distal tubule, specifically the region between the macula densa segment and the first confluence with another distal tubule, as a site where potassium is secreted at a high rate and where the rate is regulated by a number of factors that modify overall potassium excretion. Giebisch and his co-workers Malnic, Windhager, and Klose worked in the early 1960s to adapt micropuncture methods to the study of potassium transport. By collecting fluid from surface distal tubules, they showed that potassium concentration increases along the accessible distal tubule more than can be accounted for by water reabsorption (Malnic *et al.*, 1964). The results of these and subsequent experiments confirmed that the distal tubule is a site of potassium secretion (Malnic *et al.*, 1966; Wright *et al.*, 1971; Duarte *et al.*, 1971; Reineck *et al.*, 1975).

As suggested by the schematic drawing in Fig. 1, this distal tubule region

extending from the macula densa segment to the first confluence with another distal tubule is not cytologically homogenous. An early indication of functional heterogeneity in this region of the nephron came from measurements of transepithelial voltage along the length of the distal tubule. When transepithelial voltage was measured in distal segments that were carefully localized (Wright, 1971), it became clear that the magnitude of the lumen negative transepithelial voltage was greater in the more distal portion of this segment than in the more proximal part (Fig. 2). We now believe that the less negative values were measured in the subsegment now called the distal convoluted tubule, and the more negative values were measured in the subsegments now known as the connecting tubule and the initial collecting duct. We were also able to show that feeding rats a high potassium diet increased the transepithelial voltage and maintaining them on a low potassium diet for several weeks decreased the transepithelial voltage (Wright *et al.*, 1971). It has become clear that morphological heterogeneity throughout the nephron underlies such functional differences.

To give just a taste of the array of different cell types that must be considered in developing a complete understanding of the transport processes present just in the distal regions of the nephron, I have assembled some illustrations prepared by

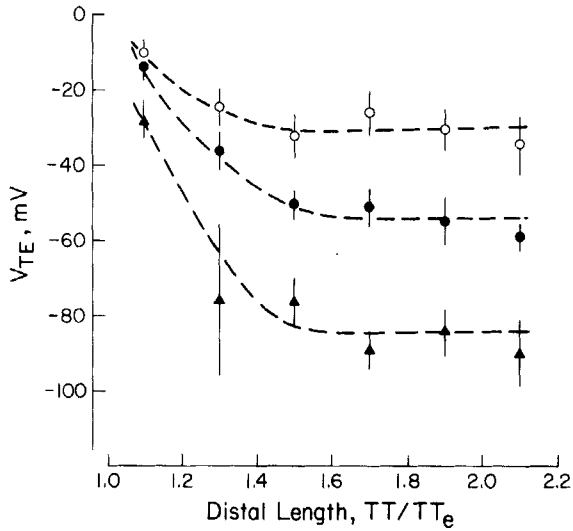

FIG. 2. Relationship between length along the distal tubule and transepithelial voltage, V_{TE}. Different symbols indicate means ±SE for pretreatment. Filled circles, normal diet; open circles, low potassium diet; filled triangles, high potassium diet. Values on x axis are an index of length along the surface distal tubule derived from the transit time of intravenously injected dye. TT, Time to punctured segment; TT_e, time to earliest appearing distal segment. [Redrawn from Wright (1974) Fig. 3.3.]

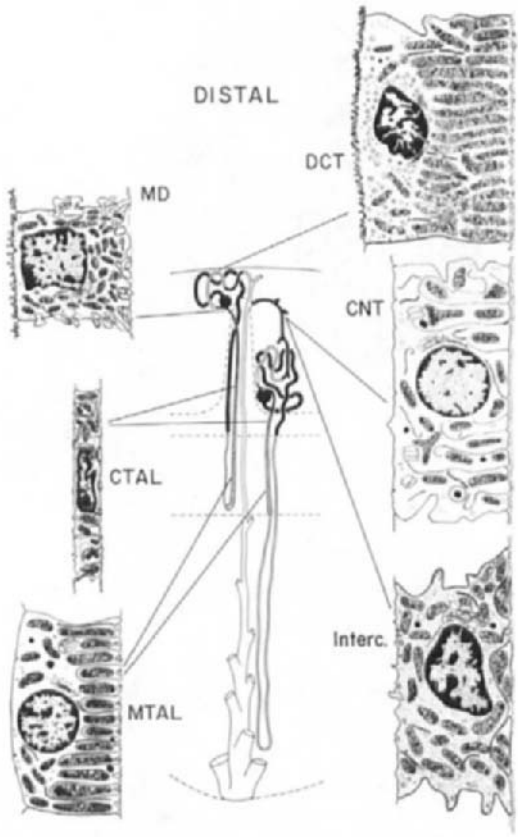

FIG. 3. Morphology of cells that make up the wall of the distal nephron. MTAL, Medullary thick ascending limb; CTAL, cortical thick ascending limb; MD, macula densa; DCT, distal convoluted tubule; CNT, connecting tubule; Interc., intercalated cell. [Modified from Kaissling and Kriz (1979), Figs. 35 and 40; and from Kaissling (1982), Fig. 1.]

Kaissling and Kriz (1979) in the course of their studies of the rabbit kidney. Cells that make up the distal tubule are shown in Fig. 3. On the left, the cells of the straight part (the medullary thick ascending limb and the cortical thick ascending limb) normally reabsorb potassium, but under some circumstances they may join the secretory ranks. Macula densa cells are difficult to study because there are only a few of them. Distal convoluted tubule cells probably reabsorb potassium, and connecting tubule cells are probably the first distal cells that regularly secrete potassium. The intercalated cells scattered along the distal tubule (Fig. 3) and along the cortical and outer medullary collecting duct (Fig. 4) are discussed by Giebisch (this volume, Chapter 8) and by Stanton (this volume, Chapter 11). The

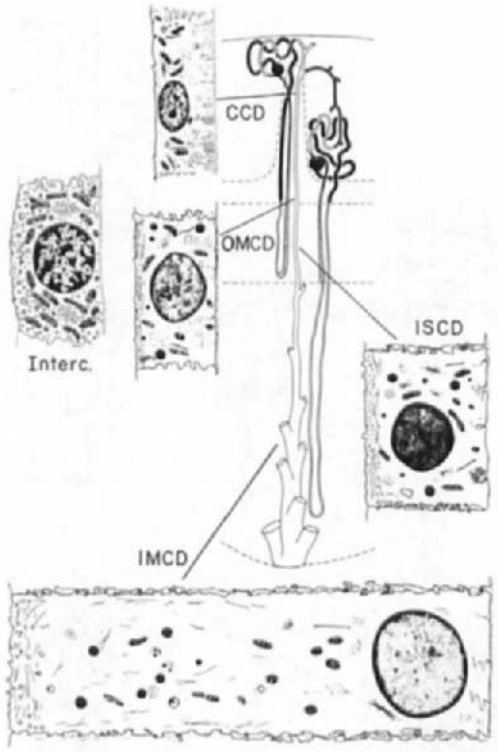

FIG. 4. Morphology of cells that make up the wall of the collecting duct system. CCD, Cortical collecting duct; OMCD, outer medullary collecting duct; Interc., intercalated cell; ISCD, inner stripe of OMCD; IMCD, inner medullary collecting duct. [Modified from Kaissling and Kriz (1979), Fig. 47; and from Kaissling (1982), Fig. 1.]

principal cells of the cortical and outer medullary collecting duct are able to secrete potassium. Their responsiveness to adrenal steroids is discussed by O'Neil (this volume, Chapter 9). The cells of the collecting duct in the inner stripe of the outer medulla permit potassium to diffuse from the collecting duct lumen into the medullary interstitium, a process that is discussed by Jamison and Müller-Suur (this volume, Chapter 7). The contribution of the cells making up the inner medullary collecting duct is not well defined.

III. DISTAL TUBULE FUNCTION

More information is available about the function of the superficial distal tubule. It is possible to relate changes in potassium secretion by cells of that

segment to changes in overall renal potassium excretion. For example, Berliner *et al.* (1950) showed that adaptive changes permit dogs to become tolerant to infusions of KCl that might otherwise be lethal. Results of their experiments are shown in Fig. 5. The left panel shows that part of this tolerance is the result of enhanced renal excretion. The open circles show increasing potassium excretion developing as a KCl solution is infused for 3 hr. The filled circles show that after the dogs were fed a high KCl diet for 2 weeks, potassium excretion increased more rapidly and to higher levels in response to a repeated infusion of KCl for 3 hr. The right panel in Fig. 5 shows results obtained by collecting fluid from surface distal tubules of rats that were treated similarly (Wright *et al.*, 1971). The open circles show that distal potassium secretion was increased modestly by 1 hr of KCl infusion. The filled circles show that in rats fed a high K diet for 4–6 weeks, distal K secretion increased rapidly to higher levels in response to the same intravenous infusion of a KCl solution. In the rats fed the high K diet, delivery of K to the end of the accessible distal tubule exceeded the rate at which K was filtered. Thus, an adaptive increase in distal potassium secretion contributes to a higher rate of renal potassium excretion, and the higher rate of potassium excretion contributes to potassium tolerance.

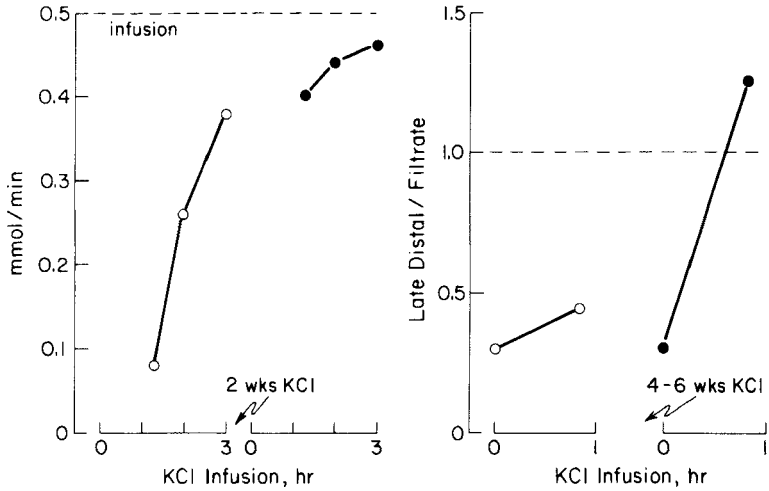

FIG. 5. Adaptive changes in whole kidney potassium excretion and in potassium secretion into distal tubules in response to a high K intake. Left panel: Renal K excretion versus time of infusion of a KCl solution. Open circles, dogs fed a normal diet; filled circles, same dogs after 2 weeks of feeding additional KCl. Broken line, rate of K infusion. (Drawn from data in Berliner *et al.*, 1950.) Right panel: K secretion into renal distal tubule versus time of infusion of a KCl solution. Open circles, rats fed a normal diet; filled circles, rats after 4–6 weeks of feeding additional KCl. Broken line, quantity of K delivered to late distal tubule equal to quantity filtered. [Drawn from data in Wright *et al.* (1971).]

A still growing fund of information derived from the efforts of many investigators tells us that the process of distal potassium secretion is regulated and plays a major role in determining known changes in renal excretion. At least four systemic variables exert a major influence on renal potassium excretion: potassium intake and total body potassium content; sodium intake and extracellular fluid volume; acid–base balance; and nonchloride anions. The mediating factors that are affected by these systemic variables and that actually act on the secretory cells of the distal nephron include the following: plasma potassium concentration, aldosterone levels, flow rate of distal tubule fluid, acidity of plasma, plasma concentrations of chloride, and nonchloride anions. Results of a large number of studies permit several generalizations about interactions between these systemic variables and mediating factors. Increases in potassium intake and in total body potassium promote potassium excretion because they cause plasma K and circulating levels of aldosterone to increase. Increases in sodium intake and in extracellular fluid volume generally promote potassium secretion (even though they suppress circulating aldosterone levels) mainly because they increase the flow rate of fluid reaching the distal nephron. Changes in acid–base status affect potassium excretion and distal secretion by modifying distal flow rate, by changing intracellular and tubule fluid pH, and by altering the anion composition of fluid in the lumen of the distal tubule. Elevated concentrations of nonchloride anions in arterial plasma tend to promote potassium excretion, apparently because of changes in the anion composition of distal tubule fluid. The separate effects of these several regulatory factors have been studied in experiments designed to allow the mediating factors to vary one at a time. Examples of such experiments follow.

In nicely designed chronic infusion experiments, Young (1982; Young and Paulsen, 1983) was able to control and vary separately changes in plasma K concentration and circulating aldosterone in order to assess their individual effects on renal potassium excretion. In Fig. 6 the left panel shows that progressively higher rates of aldosterone infusion increased daily potassium excretion (even when plasma potassium concentration was kept constant). Conversely, when adrenalectomized dogs were given constant replacement of aldosterone and potassium was infused at different rates, higher rates of potassium excretion were associated with higher plasma K concentrations. Recently, as shown in the right panel of Fig. 6, Field *et al.* (1984) found in micropuncture experiments that increasing plasma aldosterone levels, at constant plasma K concentrations, caused distal potassium secretion to increase, and that when plasma aldosterone was kept constant, increases in plasma K concentrations were associated with higher rates of distal potassium secretion.

Parallel changes in renal sodium excretion and potassium excretion have been observed in numerous studies. The results pictured in the left panel of Fig. 7, assembled from data of Berliner (1952) and of Suki *et al.* (1965), show that

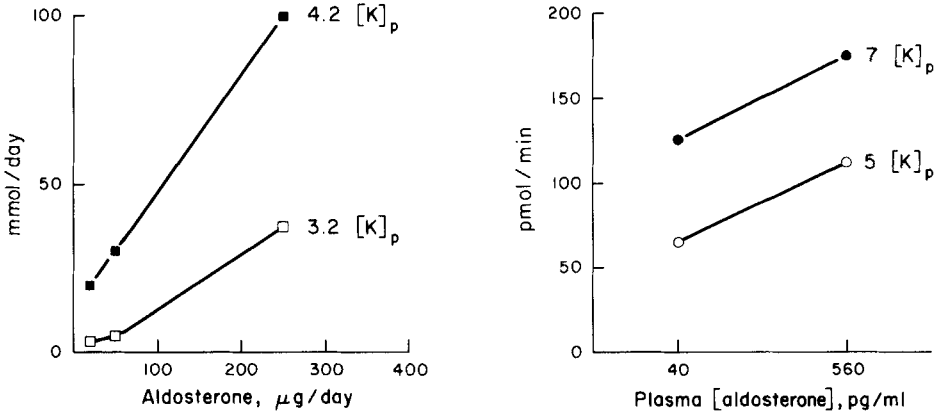

FIG. 6. Changes in whole kidney potassium excretion and in potassium secretion into distal tubules in response to increasing plasma levels of aldosterone. Left panel: Renal K excretion versus rate of aldosterone infusion into dogs. Open squares, prolonged K infusion at lower rates; filled squares, prolonged K infusion at higher rates. $[K]_p$, plasma potassium concentration. [Drawn from data in Young (1982); and Young and Paulsen (1983).] Right panel: Distal K secretion versus plasma aldosterone concentration. Open circles, K infusion at lower rates; filled circles, K infusion at higher rates. [Drawn from data in Field *et al.* (1984).]

experimental maneuvers that increase sodium excretion—extracellular fluid volume expansion with NaCl solution, administration of diuretic drugs (acetazolamide, chlorothiazide, or furosemide)—all increase potassium excretion even though plasma K concentration and circulating aldosterone are not increased.

The right panel shows results of experiments done to examine the connection between these parallel changes in sodium and potassium excretion. *In vivo* microperfusion techniques were used to assess separately the effects on distal potassium secretion of changes in tubule fluid flow rate and in luminal sodium concentration. The results in the upper figure show that when luminal sodium concentration was kept constant at either 45 or 95 m*M* and tubules were perfused at either 6 or 26 nl/min, the higher rate of perfusion was associated with a higher rate of distal potassium secretion (Good and Wright, 1979). When perfusion rate was held constant, increasing luminal Na concentration from 45 to 95 m*M* did not cause potassium secretion to increase. This flow-dependent effect on potassium secretion likely follows a nonlinear course, as suggested by the broken line. The results in the lower figure show that sufficiently low luminal Na concentrations can limit the rate of distal potassium secretion. When sodium was replaced with tetramethylammonium, and chloride concentration and fluid perfusion rate were kept constant, reducing sodium concentration from 95 to 35 m*M* had little effect on potassium secretion, whereas further reductions of Na concentration to 10 or 6 m*M* did decrease potassium secretion (Good *et al.*, 1984).

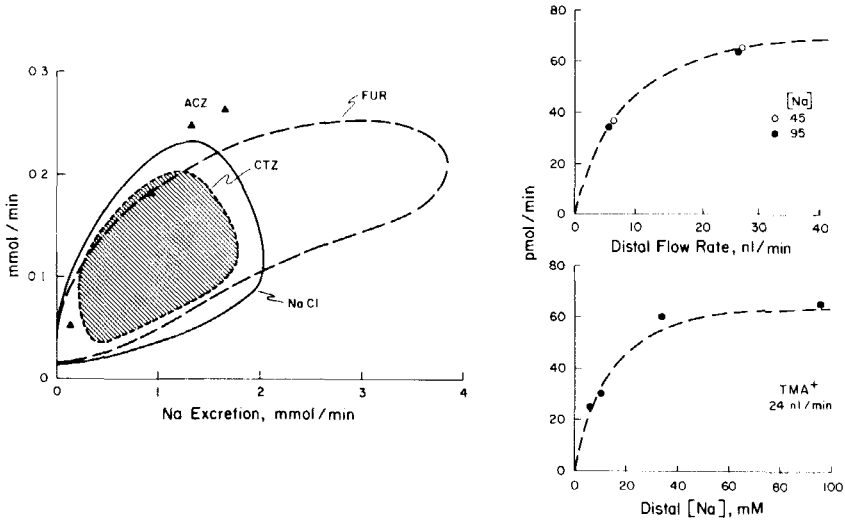

FIG. 7. Changes in whole kidney potassium excretion and in potassium secretion into distal tubules in response to changes in distal delivery and excretion of sodium. Left panel: Renal K excretion versus rate of sodium excretion stimulated by intravenous infusion into dogs of solutions containing NaCl, furosemide (FUR), chlorothiazide (CTZ), or acetazolamide (ACZ, filled triangles). [Adapted from Wright and Giebisch (1985), Fig. 5.] Upper right panel: Distal K secretion versus distal flow rate tested by *in vivo* microperfusion in rat kidneys. Perfusion fluid sodium concentration either 45 m*M* (open circles) or 95 m*M* (filled circles). Broken line is approximation. [Drawn from data in Good and Wright (1979).] Lower right panel: Distal K secretion versus sodium concentration in luminal perfusion fluid. Na replaced with tetramethylammonium (TMA); all perfusions at 24 nl/min. [Drawn from data in Good *et al.* (1984).]

Because numerous micropuncture experiments show that the Na concentration in fluid entering the distal tubule of anesthetized rats is not lower than 30 m*M*, we concluded that the main factor causing distal potassium secretion to increase during volume expansion, or diuretic drug administration, is the increase in distal flow rate and not the increase in distal sodium concentration or delivery or reabsorption that accompanies the increase in flow.

Figure 8 shows examples of the effects of acute acid–base changes on the renal handling of potassium. The left panel shows results published by Toussaint and Vererstreeten (1962). In these experiments, dogs were infused with sodium chloride, sodium bicarbonate, or ammonium chloride solutions. Changes in both plasma K and plasma pH resulted. When these variables are examined separately, it is evident not only that potassium excretion was higher when plasma K was greater, but also that potassium excretion was higher when plasma was more alkaline. The corresponding effects on distal potassium secretion were shown recently by Stanton and Giebisch (1982). Results summarized in the right panel

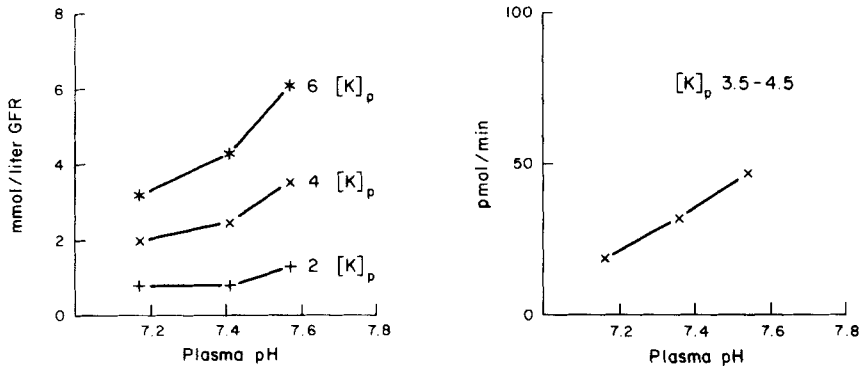

FIG. 8. Changes in whole kidney potassium excretion and in potassium secretion into distal tubules in response to changes in acid–base balance. Left panel: Renal K excretion versus plasma pH in dogs infused with NaCl (pH 7.4), $NaHCO_3$ (pH 7.5–7.6), or NH_4Cl (pH 7.1–7.2) and varying rates of KCl producing different plasma potassium concentrations $[K]_p$. Right panel: Distal K secretion versus plasma pH in rats. [Adapted from Wright and Giebisch (1985), Figs. 4 and 5.]

of Fig. 8 show that with plasma K held in a narrow range, distal potassium secretion was increased when plasma was more alkaline than normal and decreased when plasma was more acid.

These changes in potassium secretion may be caused in part by changes in intracellular pH (Giebisch, this volume, Chapter 8). It also appears that changes in the anion composition of distal tubule fluid may contribute to changes in distal potassium secretion. Berliner *et al.* (1950) compared effects of infusing chloride and nonchloride anions on potassium excretion. The left panel of Fig. 9 shows that when potassium-adapted dogs were infused with potassium chloride, potassium excretion exceeded potassium filtration by ~25%. In contrast, when dogs were infused with potassium thiosulfate, potassium excretion exceeded potassium filtration by more than 60%. It was possible that increased concentrations of nonchloride anions in plasma, or in tubule fluid, somehow stimulated the rate of distal potassium secretion. The right panel of Fig. 9 shows results of distal microperfusion experiments designed to determine whether substituting another anion for chloride in distal tubule fluid could stimulate potassium secretion. In these experiments, surface tubules in kidneys of anesthetized rats were perfused with either a chloride-containing solution or with a solution in which all chloride was replaced with sulfate. In this figure, rates of distal potassium secretion are plotted against the chloride concentration found in collected fluid. It is evident that replacing luminal chloride with sulfate causes distal potassium secretion to increase. In further experiments, we showed that this increase in potassium secretion did not depend on a change in transepithelial voltage and did not require an increase in the concentration of sulfate in either plasma or tubule fluid.

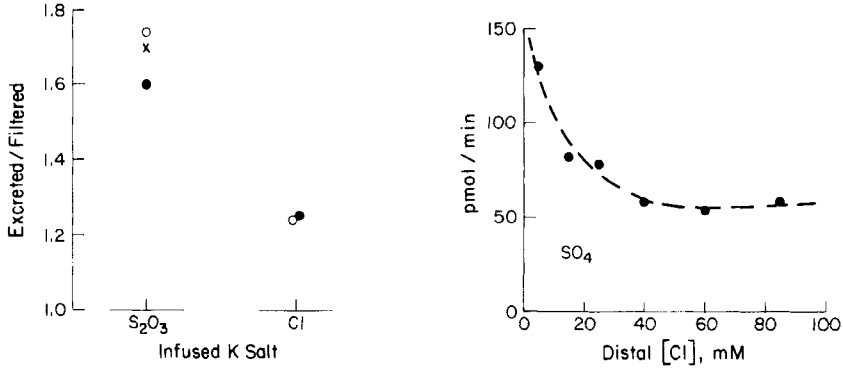

FIG. 9. Changes in whole kidney potassium excretion and in potassium secretion into distal tubules in response to chloride or nonchloride anions. Left panel: Renal K excretion by single dogs during intravenous infusion of either potassium chloride or potassium thiocyanate. [Drawn from data in Berliner *et al.* (1950).] Right panel: Distal K secretion versus chloride concentration in fluid collected from tubules perfused *in vivo*. In tubules with lower Cl concentrations chloride was replaced by sulfate. [Drawn from data in Velázquez *et al.* (1982).]

The stimulation of potassium secretion appears to occur when the concentration of chloride in luminal fluid falls to levels below 10–15 m*M*. We postulated that a mechanism linking potassium and chloride transport may be present in this nephron segment (Velázquez *et al.*, 1982).

To explore this possibility, Ellison and Velázquez have examined the effect of reducing luminal chloride concentration on unidirectional as well as net fluxes of potassium in the distal tubule (Ellison *et al.*, 1985). Their experiments employed microperfusion of surface distal tubules in anesthetized rats. When all chloride in the luminal perfusion solution was replaced with gluconate, net potassium secretion was increased. (We have also observed that replacing chloride with bicarbonate stimulates potassium secretion.) To make unidirectional flux measurements, we have used both ^{42}K and ^{86}Rb as tracers (Ellison *et al.*, 1984). We found that the secretory flux from blood to lumen is approximately six times greater than the reabsorptive flux determined from the disappearance rate of tracer from the lumen. The results of these tracer flux measurements show that reducing lumen chloride concentration stimulated potassium secretion not by decreasing the reabsorptive component of net transport, but rather by increasing the secretory component—the unidirectional flux from blood to lumen. Replacing chloride with gluconate did not change the transepithelial voltage.

Because much evidence indicates that potassium secretion involves passive diffusion of potassium from distal cells into the tubule lumen, we thought it might be possible to learn something about the way in which potassium and chloride transport are coupled if we could block the diffusive component of

potassium secretion. To do this, we sought to block conductive potassium channels with barium. Addition of 5 m*M* barium to luminal perfusion fluid decreased net potassium secretion. Barium in the lumen also decreased both unidirectional potassium fluxes—the reabsorptive flux from lumen to blood, and the larger secretory flux from blood to lumen.

Going a step further, we reasoned that if a coupled K–Cl cotransport mechanism were positioned in series with diffusive potassium transport, then barium blockade of the diffusive transport step should interfere with the effect of low lumen chloride to stimulate potassium secretion. Conversely, if a coupled K/Cl cotransport mechanism operates in parallel with diffusive potassium transport—both mechanisms being present in the luminal membrane—then barium blockade of potassium channels would not be expected to interfere with the stimulation of potassium secretion resulting from lowering luminal chloride concentration. We therefore performed experiments in which perfusion fluid chloride was replaced with gluconate in the presence of 5 m*M* barium. Net potassium secretion was increased when lumen chloride was reduced. The magnitude of the increase in the presence of barium was not different from the size of the increase previously seen in the absence of barium. Again, net potassium secretion was increased not because low lumen chloride decreased the lumen to blood flux, but rather because reducing lumen chloride concentration stimulated the unidirectional secretory flux. We believe these results point to the operation of a K–Cl cotransport mechanism that is situated in the luminal membrane of distal cells and that responds to reductions in lumen chloride concentration by increasing movement of potassium from cell to lumen.

IV. CONCLUSION

The idea that a distal secretory process provides the major route by which potassium gains access to the urine and is ultimately excreted has been confirmed in several respects. The rate of distal secretion is variable and is changed from near zero to rates exceeding the potassium filtration rate by factors that change urinary excretion in the same direction. Potassium is not totally reabsorbed by the proximal tubule and by the ascending limb of the loop of Henle, but the quantity of potassium left unreabsorbed at the beginning of the distal secretory region does not vary over as wide a range as the quantity reaching the collecting duct or the final urine.

In the years since Berliner and his co-workers first proposed the central role for distal potassium secretion, it has been possible to identify a satisfyingly large number of factors able to modify the secretory process. As we come to know the individual contributions of each of these factors, we are better able to understand what the kidney does with potassium and how it does it.

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Chapter 7

Potassium Recycling

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I. INTRODUCTION

On the basis of clearance studies in man and animals summarized by Berliner (1961), the concept emerged that filtration and excretion of potassium are independent of each other—filtered potassium is nearly completely reabsorbed, while potassium excreted in the urine is derived from secretion at a site beyond the tubule segment in which potassium is reabsorbed. This hypothesis was supported by stop-flow experiments that suggested a distal secretory site (Malvin *et al.*, 1958) and by microinjection studies with ^{42}K that indicated nearly complete reabsorption of filtered potassium (Morel, 1955). Micropuncture experiments by Malnic *et al.* (1964, 1966a,b, 1971) were decisive, demonstrating in the rat that at least 50% of the filtered potassium is reabsorbed in the proximal tubule and only 10% reaches the early distal tubule in steady-state conditions. Moreover, the excreted potassium could be accounted for—in fact, sometimes more than accounted for—by tubule secretion along the distal nephron accessible on the cortical surface. As described elsewhere in this volume, micropuncture of the superficial nephron by Malnic *et al.* (1964, 1966a,b, 1971), Wright *et al.* (Good and Wright, 1979; Peterson and Wright, 1977; Wright, 1977; Wright and Giebisch, 1978; Wright *et al.*, 1971, 1981), and Giebisch *et al.* (Duarte *et al.*, 1971; Field *et al.*, 1984; Giebisch and Stanton, 1979), among others, has also elucidated the factors regulating the reabsorptive and secretory processes in the proximal convoluted tubule and accessible distal tubule. *In vitro* perfusion of

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single isolated fragments of the distal convoluted and cortical collecting tubule dissected from the rabbit kidney by Grantham *et al.* (1970), Gross *et al.* (1975), and others (see Jamison *et al.*, 1982) has confirmed and extended the micropuncture findings. The recent structural–functional correlations in the connecting tubule, initial cortical collecting tubule, and collecting tubule during adaptation to a chronic potassium load have added another dimension (Stanton *et al.* 1981).

Over the past decade, however, other findings have accumulated that have indicated that the transepithelial movement in segments of the juxtamedullary nephron is not in accord with the concept just described (Arrascue *et al.*, 1981; Battilana *et al.*, 1978; de Rouffignac and Morel, 1969; de Rouffignac *et al.*, 1973; Dobyan *et al.*, 1979, 1980; Elalouf *et al.*, 1985; Grantham *et al.*, 1974; Higashihara and Kokko, 1985; Imai, 1984; Jamison and Kriz, 1982; Jamison *et al.*, 1976, 1982; Milanes and Jamison, 1985; Müller-Suur and Jamison, 1984; Stokes, 1982a,b; Sufit and Jamison, 1982, 1983; Wasserstein and Agus, 1983; Wong *et al.*, 1981; Work *et al.*, 1982). Instead, the evidence strongly suggests that some of the potassium leaving the collecting tubule in the cortex is reabsorbed in the medullary collecting tubule, trapped in the medullary interstitium by countercurrent exchange, and secreted into the pars recta of the superficial nephron and pars recta and descending limb of the juxtamedullary nephron; i.e., that potassium undergoes recycling in the renal medulla (Battilana *et al.*, 1978). The purposes of this review are to summarize the evidence for potassium recycling and to suggest a role that recycling might play in the regulation of urinary potassium excretion.

II. POTASSIUM RECYCLING

In young rats, the tip of the renal papilla protrudes into the pelvic ureter and can be exposed by excision of the ureter to gain access to the end-descending limb of the long Henle's loops of the juxtamedullary nephron. Using the micropuncture technique, we demonstrated that fractional delivery of potassium to the end of the juxtamedullary descending limb is normally equal to or slightly greater than the filtered load of potassium (Jamison *et al.*, 1976). Treatment of animals with benzolamide, a carbonic anhydrase inhibitor that decreases the reabsorption of sodium, bicarbonate, and water in the proximal convoluted tubule, increased potassium delivery to the end-descending limb to a value equivalent to 177% of the filtered load of potassium, which established unequivocally that potassium is secreted (either passively or actively) into the juxtamedullary nephron upstream to the hairpin turn (Jamison *et al.*, 1976). Potassium deliveries clearly exceeding the filtered load were also observed in animals chronically fed a high potassium

diet (Battilana *et al.*, 1978), or acutely infused with potassium (Arrascue *et al.*, 1981). We inferred from the high potassium concentrations in vasa recta plasma (Battilana *et al.*, 1978) and in the medullary interstitium (Bulger *et al.*, 1981) that the secreted potassium is supplied by the medullary interstitium. On the other hand, if the medullary interstitial potassium concentration is diminished by furosemide (Jamison *et al.*, 1976), chronic water diuresis (Battilana *et al.*, 1978), or dietary potassium deprivation (Dobyan *et al.*, 1979), potassium delivery to the end-descending limb is reduced. Figure 1 summarizes the findings obtained from several laboratories using micropuncture of the long loops. Fractional delivery of potassium to the end-descending limb is plotted as a function of

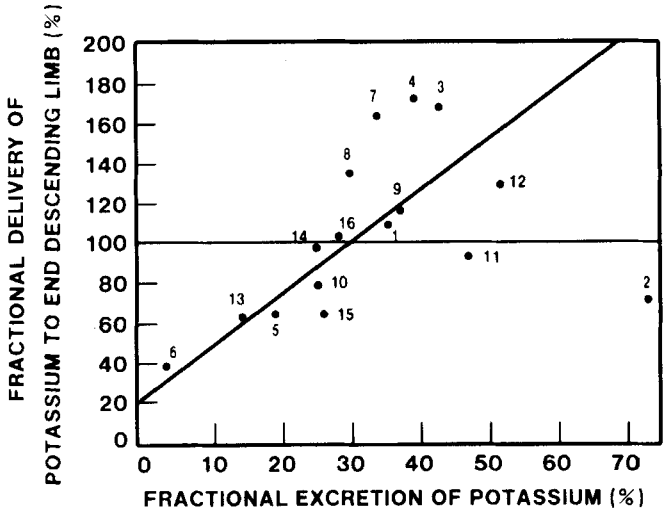

FIG. 1. Summary of micropuncture studies of potassium recycling. Fractional delivery of potassium to the end-descending limb of the juxtamedullary nephron (in percentage) is plotted on the ordinate as a function of fractional excretion of potassium (in percentage) on the abscissa. The numbers beside each symbol correspond to the following experimental conditions and investigators: (1) normal, Jamison *et al.*, 1976; (2) furosemide, Jamison *et al.*, 1976; (3) benzolamide, Jamison *et al.*, 1976; (4) chronic K load, Battilana *et al.*, 1978; (5) chronic K load + amiloride, Battilana *et al.*, 1978; (6) low K diet, Dobyan *et al.*, 1979; (7) acute K load, Arrascue *et al.*, 1981; (8) normal, Roy *et al.*, 1982; (9) acute metabolic acidosis, Roy *et al.*, 1982; (10) acute respiratory acidosis, Roy *et al.*, 1982; (11) acute metabolic alkalosis, Roy *et al.*, 1982; (12) sulfate infusion, Roy *et al.*, 1982; (13) adrenalectomy + dexamethasone, Higashihara and Kokko, 1985; (14) Adx + Dex + aldosterone, Higashihara and Kokko, 1985; (15) "hormone deprived," Elalouf *et al.*, 1985; (16) "hormone deprived" + dDAVP, Elalouf *et al.*, 1985. The regression line was calculated from all conditions except (2 and 11), for reasons discussed in the text, and (3), because benzolamide changes potassium delivery to the beginning of the descending limb. The horizontal line indicates 100% fractional delivery to the end-descending limb.

fractional excretion of potassium. This relationship, originally observed by Battilana *et al.* (1978), forms the basis for the hypothesis of medullary recycling. With two exceptions, furosemide administration (Jamison *et al.*, 1976) (number 2 in Fig. 1) and metabolic alkalosis (Roy *et al.*, 1982) number 11 in Fig. 1), fractional delivery of potassium to the end-descending limb is equal to or greater than 100% when fractional potassium excretion is 30% or greater.

The relationship expressed in Fig. 1 could be explained by either of two general hypotheses (Battilana *et al.*, 1978). One is that potassium delivered to the end-descending limb is reabsorbed from the thin ascending limb in the inner medulla and directly shunted to the medullary collecting duct to be secreted into the urine. The other hypothesis is that, conversely, potassium is reabsorbed from medullary collecting duct, trapped in the interstitium, and reenters the juxtamedullary nephron upstream to the end-descending limb. (Implicit in the latter hypothesis is the assumption that the urinary excretion of potassium is an index of the delivery of potassium to the beginning of the medullary collecting duct.) The correct choice between these alternatives is revealed by measures which selectively reduce potassium secretion beyond the loop of Henle in the connecting tubule and cortical collecting tubule, and would therefore reduce the source of potassium for medullary recycling, but do not act on the renal tubule transport of potassium upstream to the end-descending limb. If the first hypothesis is correct, there should be no effect of these measures on the delivery of potassium to the end-descending limb. If the second hypothesis is correct, then delivery of potassium to the end-descending limb should decrease as the fractional excretion of potassium falls. Potassium delivery to the medullary collecting duct was reduced by amiloride administration to chronically potassium-loaded rats (number 5 in Fig. 1) (Battilana *et al.*, 1978), a brief feeding of a potassium-free diet (number 6 in Fig. 1) (Dobyan *et al.*, 1979), and aldosterone deficiency (number 13 in Fig. 1) (Higashihara and Kokko, 1985). In each case, fractional potassium delivery to the end-descending limb was reduced to 65% or less. Note in particular the effect of amiloride (Battilana *et al.*, 1978) and aldosterone deficiency (Higashihara and Kokko, 1985), both of which inhibit potassium secretion in the connecting tubule-cortical collecting tubule segment, but have no known effect on potassium movement in the proximal tubule or pars recta (Duarte *et al.*, 1971) (and if either amiloride or aldosterone deficiency did have an effect on these segments, it would have to be to *enhance* potassium reabsorption to account for the decline in potassium delivery to the end-descending limb). In animals deprived of potassium, fractional potassium delivery was diminished to a value essentially the same as the fractional delivery of sodium to the end-descending limb. Since fractional delivery of potassium from the proximal tubule is known to be approximately the same as that of sodium and little or no transepithelial addition of sodium normally occurs in the descending limb, it was

as if potassium secretion were completely abolished upstream to the descending limb by a potassium-free diet (Dobyan *et al.*, 1979). The so-called hormonally deprived rat that lacks calcitonin, parathyroid hormone, insulin, and antidiuretic hormone, also exhibited a low potassium delivery to the end-descending limb (number 15 in Fig. 1), which was corrected by dDAVP, a synthetic antidiuretic agonist of antiduretic hormone (number 16 in Fig. 1). It was demonstrated that dDAVP stimulated potassium secretion in the distal nephron of the hormonally deprived rat (Elalouf *et al.*, 1985). The foregoing findings and the other findings illustrated in Fig. 1 support the second hypothesis, the so-called potassium recycling hypothesis formulated by Battilana and his colleagues (1978). As mentioned, two apparent exceptions to this hypothesis (Fig. 1) are furosemide administration (Jamison *et al.*, 1976) and acute metabolic alkalosis (Roy *et al.*, 1982). We will return to these apparent exceptions below.

III. PREDICTIONS OF THE POTASSIUM RECYCLING HYPOTHESIS

The usefulness of a hypothesis depends upon the extent to which it can be tested; it remains unmodified as long as it passes each test. One test of the potassium recycling hypothesis is to determine whether it is consistent with previous work. The recycling hypothesis is entirely consistent with previous clearance and stop-flow studies, since potassium reabsorption still precedes secretion, although in a modified sequence: reabsorption in the proximal tubule, secretion in the pars recta and descending limb, reabsorption in the ascending limb, and secretion beyond the ascending limb. Is the recycling hypothesis consistent with previous micropuncture work? Again, with the reservations expressed above, the answer is in the affirmative. As early as 1969, micropuncture experiments in the *Psammomys* by de Rouffignac and his co-workers (de Rouffignac and Morel, 1969; de Rouffignac *et al.*, 1973) pointed strongly to the existence of medullary recycling of potassium and recycling of other cations as well. Most investigations showing that potassium is secreted along the superficial distal nephron have disclosed considerable variability between the fraction of potassium remaining at the beginning of the collecting tubule and that excreted in the urine. In micropuncture of the medullary collecting duct accessible in the exposed papilla, there is also considerable variability between base and tip potassium mass flows (Reineck *et al.*, 1978). There seems to be reversal of net potassium transport as one descends from the cortical to medullary collecting tubule—secretion predominating in the cortical segments, reabsorption occurring somewhere along the medullary collecting duct. An important factor may be the fluid flow rate in this regard. Under circumstances of high urinary potassium

excretion and low urinary flow, the potassium concentration in the collecting tubule would reach very high levels unless there were some potassium reabsorption in the medullary collecting duct, as pointed out by Reineck *et al.* (1978).

The next tests of the recycling hypothesis are of some of its predictions. Three predictions of the recycling hypothesis which were tested were the following: (1) potassium is reabsorbed in the medullary collecting duct; (2) potassium is secreted in the proximal convoluted or straight tubule; and (3) potassium is secreted in the descending limb of the juxtamedullary nephron.

IV. MEDULLARY COLLECTING DUCT

The precise location and extent of potassium reabsorption by the medullary collecting duct under various conditions of potassium intake are unknown. Studies of the isolated perfused cortical collecting duct *in vitro* have demonstrated active potassium secretion (Grantham *et al.*, 1970; Gross *et al.*, 1975). Evidence of potassium reabsorption by the same segment under any of the conditions studied is lacking. Compared to the cortical collecting tubule, however, the outer medullary collecting duct has strikingly different transport characteristics, according to Stokes (1982b). The latter segment behaves as a passive membrane to sodium and potassium; i.e., the transepithelial movement of either cation is determined by the permeability and transepithelial electrochemical gradient, respectively, for each ion. Although the permeability of the outer medullary collecting duct to potassium is relatively low, the potassium concentration in collecting tubule fluid entering the beginning of the outer medullary collecting duct is likely to be much greater than the potassium concentration in the interstitium surrounding the outer medullary collecting duct. Thus, the outer medullary collecting duct (in particular the inner stripe segment, Stokes, 1982b) is a prime candidate for the site of potassium reabsorption *in vivo*. While it may seem incongruous to have a segment that permits passive transepithelial movement of potassium located just downstream to the cortical collecting tubule in which energy has been expended to drive potassium into the lumen to high concentrations, the juxtaposition of the two segments is entirely consistent with the recycling hypothesis.

Microcatheterization and micropuncture studies of the inner medullary collecting tubule have shown no consistent net potassium reabsorption in animals fed a normal potassium diet (Giebisch and Stanton, 1979; Good and Wright, 1979; Grantham *et al.*, 1970, 1974; Greger and Schlatter, 1981; Gross *et al.*, 1975; Hayslett *et al.*, 1980). In animals loaded with potassium there is, if anything, a slight tendency to potassium secretion (Hayslett *et al.*, 1980). Potassium is reabsorbed in the inner medullary collecting duct in animals fed a low potassium diet. These findings, while they do not rule out the inner medullary collecting

duct as a potential site for potassium reabsorption under normal circumstances, do suggest that the outer medullary collecting duct is the most likely site in which potassium recycling is initiated.

V. PROXIMAL CONVOLUTED TUBULE AND STRAIGHT TUBULE

Most micropuncture investigations of the proximal convoluted tubule have shown that potassium reabsorption is proportional to that in sodium and water. Recent studies, however, have shown that the tubular fluid-to-plasma potassium ratios exceed unity toward the end of the accessible proximal convoluted tubule. In fact, they rise to 1.4 at the end of the accessible proximal tubule of the *Perognathus* (Braun *et al.*, 1981). Kaufman and Hamburger found in studies of the rabbit proximal convoluted tubule isolated and perfused *in vitro* that about half of the segments exhibit net potassium reabsorption and the other half net potassium secretion. Ouabain diminished both potassium reabsorption and potassium secretion. In a later study, however, they found the transepithelial potassium reabsorption was entirely passive and dependent on the concentration difference across the epithelium (Kaufman and Hamburger, 1985). Of note, the potassium permeability was higher in the juxtamedullary proximal convoluted tubule than in the superficial proximal convoluted tubule (Kaufman and Hamburger, 1985).

Grantham and his colleagues (1974) were the first to observe potassium secretion in the pars recta, but in rather unusual experimental conditions. The pars recta of a rabbit was isolated and one end attached to a pipette and immersed in a bathing medium. The other end was crimped and *p*-aminohippuric acid (PAH) was added to the bath. The secretion of PAH into the pars recta induced net fluid secretion. Potassium secretion was observed in these conditions and to a greater extent than could be accounted for by the net fluid secretion. More recently, Work *et al.* (1982) and Wasserstein and Agus (1983) independently demonstrated net potassium secretion under conditions in which fluid reabsorption occurred in the S_2 and S_3 segments of superficial and juxtamedullary nephrons. Although both groups inferred that potassium secretion was active, it was inhibitable by ouabain in Wasserstein's study, but not in Work's. Both groups found that when even a modest favorable bath-to-lumen gradient (5 m*M*) was imposed, potassium secretion was markedly enhanced. In agreement with findings of the proximal convoluted segments (Kaufman and Hamburger, 1985), the potassium permeability of the juxtamedullary pars recta was higher than that of the superficial pars recta, which would facilitate potassium secretion, especially in the juxtamedullary nephron, if the outer medullary interstitium had even a slightly elevated potassium concentration from potassium recycling.

VI. DESCENDING LIMB OF THE JUXTAMEDULLARY NEPHRON

Work *et al.* (1982) calculated that even under the most favorable conditions, the increase in potassium mass flow entering the descending limb would still account for less than half of the mass flow at the end of the juxtamedullary descending limb (Jamison *et al.*, 1976). This strongly suggests that potassium secretion must also occur in the descending limb. Jamison and his colleagues (1976) constructed a theoretical model incorporating appropriate conditions concerning descending limb fluid and medullary interstitium and the potassium permeability properties of the rabbit descending limb (Rocha and Kokko, 1973). Under these conditions, potassium entry along the thin descending limb was sufficient to account for the mass flow of potassium at the end of the descending limb (Jamison *et al.*, 1976). It would not account for the potassium mass flow observed after chronic (Battilana *et al.*, 1978) or acute (Arrascue *et al.*, 1981) potassium loading, however. Recently, Imai (1984) examined the proximal portion of the descending limb of the juxtamedullary nephron of the rat and found that it has a higher potassium permeability than the rabbit thin descending limb, which may be sufficient to account for the potassium entry observed under potassium loading conditions. It would be interesting to determine if potassium secretion by the pars recta or descending limb *in vitro* is enhanced by prior acute or chronic potassium loading and, if so, how.

VII. PHYSIOLOGICAL ROLE OF POTASSIUM RECYCLING

The hypothesis of medullary recycling of potassium seems to have passed all tests so far. Though much further work needs to be done, the existence of recycling no longer can be seriously doubted. In the past 3 years, several groups (Milanes and Jamison, 1985; Müller-Suur and Jamison, 1984; Stokes, 1982a,b; Sufit and Jamison, 1983) have begun to consider the role of potassium recycling. It seemed reasonable to assume that potassium recycling has something to do with regulating urinary excretion of potassium. In the steady state, however, it is most unlikely that potassium recycling plays a role in regulating potassium excretion. The reduction in mass flow of potassium along the collecting duct owing to potassium reabsorption would be counterbalanced by the potassium captured by the juxtamedullary nephron and returned to the collecting duct and thus neither reduce nor augment urinary potassium excretion. By analogy, urea, which also undergoes recycling in the renal medulla (Jamison and Kriz, 1982), is excreted transiently at a greater rate during the transition from antidiuresis to

water diuresis and transiently at a lower rate during the reverse transition, but once the steady state is reached, the urea excretion rate is the same in water diuresis as it is in antidiuresis and reflects the urea generation rate (Jamison and Kriz, 1982). With respect to potassium, one is usually not in a steady state, however. The kidney is either charged with excreting an ingested load of potassium or else conserving potassium between meals. In the response to a potassium load, potassium recycling might augment urinary potassium excretion either by capturing potassium reabsorbed in the outer medullary collecting duct and returning it to the collecting duct by way of the juxtamedullary nephron (rather than allow the cation to return to the systemic circulation) or by stimulating potassium secretion in the distal tubule.

In 1982, Stokes (1982a) examined the effect of imposing transepithelial potassium concentration gradients on the rabbit medullary thick ascending limb perfused *in vitro*. If the perfusate concentration was raised to 25 m*M* while the bath concentration was 5 m*M*, potassium reabsorption was markedly enhanced, although the mass flow of potassium leaving the end of the thick ascending limb was still elevated. The most striking finding, however, was the virtual abolition of sodium reabsorption, from 102 to 13 pEq/mm tubule length/min. In contrast, chloride reabsorption was decreased only by 20%; in effect, potassium substituted for sodium as the cation accompanying chloride reabsorption across the medullary thick ascending limb. Under the opposite condition, when the bath concentration of potassium was elevated to 25 m*M* while the perfusate potassium concentration was 5 m*M*, potassium reabsorption was reversed to potassium secretion, 33 pEq/mm tubule length/min, which increased the mass flow of potassium from the distal end of the tubule. In this case, it was chloride reabsorption that was nearly abolished—from 114 to 5 pEq/mm tubule length/min. Sodium reabsorption was reduced only from 92 to 57. In this circumstance, potassium secretion was involved in an apparent ion-for-ion exchange with sodium reabsorption. These findings by Stokes suggest that the medullary thick ascending limb acts as a transducer, converting an effect of medullary potassium recycling to increase the potassium concentration of the medullary interstitium or thick ascending limb fluid into an inhibitory effect on sodium chloride reabsorption by the thick ascending limb. By this means, potassium recycling could augment potassium mass flow in the connecting tubule and cortical collecting tubule in three ways (Stokes, 1982a):

1. Increased potassium flow from the medullary thick ascending limb.
2. Increased fluid flow to the connecting tubule which stimulates potassium secretion. The increased flow of fluid results from the decline in medullary osmolality secondary to the inhibition of NaCl reabsorption in the medullary thick ascending limb. The consequent decrease in the water extraction from the

descending limb would enhance the fluid flow entering the thick ascending limb and distal tubule.

3. Increased delivery of sodium chloride to the cortical collecting duct which stimulates potassium secretion.

We have performed three experiments in our laboratory (Milanes and Jamison, 1985; Müller-Suur and Jamison, 1984; Sufit and Jamison, 1982) to test Stokes's hypothesis.

First, normal rats were studied by Carl Sufit (Sufit and Jamison, 1983) before and after an acute infusion of potassium chloride to determine whether potassium recycling enhances the delivery of potassium, sodium, and water from the thick ascending limb to the distal tubule of the superficial nephron. Samples of fluid were obtained by micropuncture from the end-accessible proximal tubule and the beginning of the accessible distal tubule before and after acute potassium loading to determine reabsorption by the intervening segment (the loop of Henle) and delivery to the distal tubule. Potassium reabsorption by the loop of Henle fell from 75% to 58% after KCl infusion. Fractional delivery of potassium to the beginning of the distal tubule increased from 12% to 26% (Fig. 2). Assuming

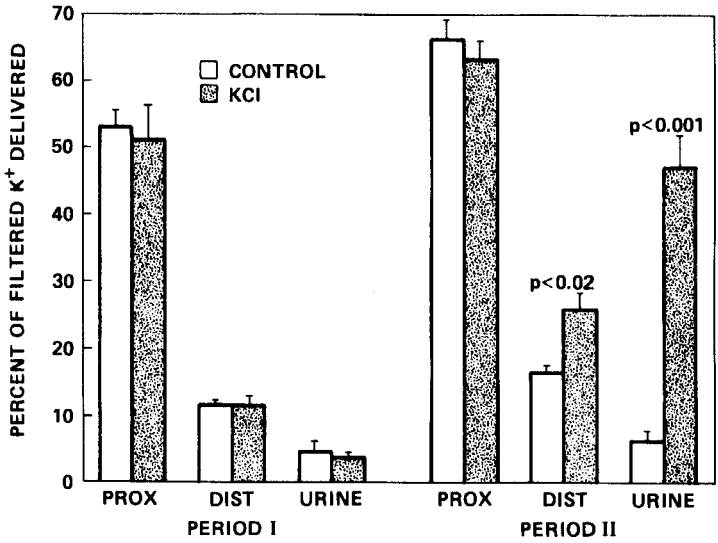

FIG. 2. Fraction of filtered potassium delivered (in percentage) to end of accessible proximal tubule (Prox), beginning of accessible distal tubule (DIST), and final urine in Periods I and II. Control animals were infused with isotonic saline throughout experiment. KCl rats were also infused with KCl after the end of Period I. Statistical comparisons are between control and KCl groups. [From Sufit and Jamison (1982). Reproduced with permission.]

these findings are representative of potassium delivery to the distal tubule of all nephrons, the potassium delivery was equivalent to half of the urinary excretion rate of potassium. There was no significant change in the reabsorption of sodium or water by the loop of Henle, however, although the sodium concentration in fluid entering the distal tubule did rise significantly. We reasoned that sodium reabsorption in the medullary thick ascending limb may also have been inhibited, but that the cortical thick ascending limb was able to reabsorb the excess sodium chloride delivered to it (Burg and Bourdeau, 1978), thus preventing a rise in sodium chloride delivery to the beginning of the superficial distal tubule.

Therefore, in a second experiment, Carmen Milanes (Milanes and Jamison, 1985) employed the same protocol in a model of chronic renal failure partly on the premise that the presumed inhibition of sodium chloride reabsorption in the thick ascending limb might be more readily detectable. The right kidney of the rat was removed and branches of the left renal artery were ligated which resulted in a functioning remnant kidney. One week later and after one day of a potassium-free diet, the rats were studied before and after acute loads of potassium chloride which increased the urinary potassium excretion from 5% to 50%. Potassium reabsorption by the loop of Henle fell from 64% to 48%. Fractional delivery of potassium to the beginning of the distal tubule increased from 17% to 35% (Fig. 3), which was equivalent to three-quarters of the urinary potassium excretion of the superficial nephron. Again, however, there was no significant increase in either the fractional delivery of sodium or water to the beginning of the accessible distal tubule. Sodium chloride delivery to the beginning of the distal tubule of the juxtamedullary nephron might have been increased, since this nephron lacks a cortical thick ascending limb (Jamison and Kriz, 1982), but this segment is inaccessible to micropuncture.

An assessment of reabsorption in the loop of Henle by subtracting delivery to the beginning of the accessible distal tubule from that to the end of the accessible proximal tubule is inevitably inexact because of variability in flows among tubule segments and the fact that the proximal tubule and distal tubule of different nephrons, rather than of the same nephron, are sampled. A significant inhibition of NaCl and water reabsorption might conceivably have gone undetected. A third experiment, designed to avoid these limitations, was undertaken by Müller-Suur and employed micropefusion of the loop of Henle *in vivo* (Müller-Suur and Jamison, 1984). The end-accessible proximal tubule was punctured and flow of tubule fluid from upstream segments was stopped by the injection of an immobile wax block. The loop of Henle was perfused at a predetermined rate by an artificial fluid whose composition resembled that of fluid in the pars recta. The beginning of the accessible distal tubule of the perfused nephron was punctured, and the perfusate was collected before and after an acute potassium load. Preliminary findings disclosed that under these conditions, fractional reabsorption of

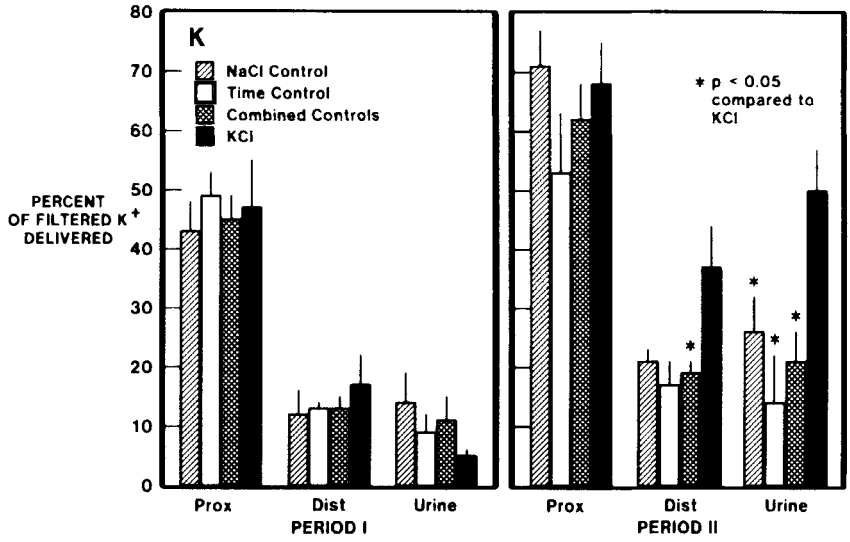

Fig. 3. Fraction of filtered potassium delivered (in percentage) to end of accessible proximal tubule (Prox), beginning of accessible distal tubule (Dist), and final urine in Periods I and II. NaCl control animals received NaCl at a rate of 72 μmol/min/kg body weight added to the saline infusion in Period II. Time control animals had no solute added to the saline infusion in Period II. Combined control illustrates the results of the NaCl and time control groups combined. The KCl group received KCl at a rate of 72 μmol/min/kg body weight in the second period. The vertical bars represent ±1 standard error. Statistical comparisons are between each control group and the KCl group. [From Milanes and Jamison (1985). Reproduced with permission.]

sodium chloride as well as that of potassium was reduced. Water reabsorption, however, was not diminished. (Control studies to exclude time as a variable were not done in these preliminary experiments, however.) By controlling the composition and flow of fluid entering the loop of Henle, an apparent inhibitory effect of acute potassium loading on sodium chloride reabsorption in the thick ascending limb was uncovered, confirming the second prediction of Stokes's hypothesis. Fluid flow entering the distal tubule, however, did not increase.

The mechanism by which an elevated potassium concentration on either side affects changes in sodium chloride reabsorption in the thick ascending limb is not understood, but it is tempting to suggest that the effect may be mediated by the liminal carrier which requires one sodium, one potassium, and two chloride ions to move from the lumen to the cell interior (Greger and Schlatter, 1981).

VIII. FUROSEMIDE, ACUTE METABOLIC ALKALOSIS, AND UREA

Returning now to Fig. 1, consider the two conditions in which the delivery of potassium to the end-descending limb did not rise above 100% when potassium excretion exceeded 30% (Jamison *et al.*, 1976; Roy *et al.*, 1982). If potassium recycling inhibits sodium chloride reabsorption by the thick ascending limb, then conditions which themselves independently reduce sodium chloride reabsorption in the thick ascending limb might disrupt the relationship depicted in Fig. 1. An obvious example is furosemide administration, which profoundly inhibits sodium chloride reabsorption in the thick ascending limb (Burg and Bourdeau, 1978). An inhibitory effect of potassium on sodium chloride reabsorption would therefore by obscured by furosemide. Similarly, in acute metabolic alkalosis, salt depletion and the reduced chloride delivery would reduce NaCl reabsorption by the thick ascending limb.

In 1948, Mudge, Foulks, and Gilman studied osmotic diuresis in dogs, produced by the infusion of urea, and observed that the excretion of potassium was greater than could be accounted for by glomerular filtration, indicating potassium secretion by the renal tubule. In contrast, Wesson, Anslow, and Smith (1948), who were also studying osmotic diuresis in dogs, but infused mannitol instead of urea, did not find evidence of potassium secretion, although they did later when they repeated their experiments with infused urea. The difference between the effects on potassium excretion between mannitol and urea was not explicable at the time. Recently, Wong *et al.* undertook a micropuncture study of dogs subjected to a mannitol (Wong *et al.*, 1979) or urea (Wong *et al.*, 1982) osmotic diuresis and discovered that after urea but not mannitol infusion, the fraction of filtered potassium delivered to the beginning of the distal tubule, 113%, was greater than the delivery of potassium to the end of the superficial proximal tubule, 73%, indicating net potassium secretion in the loop of Henle. It seems reasonable to infer that the greater increase in urinary potassium excretion observed in urea diuresis than in mannitol diuresis may reflect the fact that urea, but not mannitol, caused potassium secretion in the loop of Henle, which, coupled to potassium secretion further downstream, resulted in a higher potassium excretion rate. Why urea should induce potassium secretion in the loop of Henle remains to be determined.

IX. SUMMARY

In summary, potassium excretion is governed primarily by the regulation of potassium secretion in the distal nephron and collecting duct in accordance with

the classic model. Recent findings that have formed the basis for this review, however, suggest that renal potassium transport is a more complex process involving functional heterogeneity between the superficial and juxtamedullary nephron and among successive segments of the same nephron. From analysis of the mass flow of potassium along the superficial and juxtamedullary nephrons, we have proposed that potassium is recycled in the medulla; i.e., potassium is reabsorbed from the outer medullary collecting duct (and normally also from the thick ascending limb) and secreted into the pars recta and thin descending limb of Henle's loop. Potassium delivery from the thick ascending limb added to the potassium secretion in the connecting tubule and cortical collecting tubule transiently augments total potassium mass flow. We further suggest, in accord with Stokes (1982a), that recycling of potassium inhibits NaCl reabsorption in the medullary thick ascending limb and that the increased sodium chloride delivery (perhaps primarily in the juxtamedullary nephron) stimulates the secretion of potassium in the cortical collecting tubule. In effect, medullary recycling of potassium initiates a postive feedback which accelerates the excretion of an acute potassium load. It seems likely that flow of fluid reaching the distal tubule is also enhanced, but since this is secondary to a reduced axial osmotic gradient in the renal medulla, it is likely to be much more pronounced in the long descending limb of the juxtamedullary nephron than in the short descending limb of superficial nephron and may explain why an increased fluid flow was not observed in the superficial nephron.

If medullary recycling of potassium plays a role in the excretion of acute potassium load, this might explain why in certain kidney diseases there is reduced ability to excrete acute potassium loads despite a glomerular filtration rate which is more than adequate. Examples of kidney disease in which this phenomenon has been observed are cited elsewhere (Milanes and Jamison, 1985). Suffice it to suggest that while in some of these diseases a reduced secretion of renin and aldosterone has been offered as the explanation for the impaired potassium excretion, other findings suggest that a disordered renin–aldosterone system does not adequately account for the reduced potassium excretion in these diseases; they point instead to a primary tubule defect in potassium secretion. It is noteworthy that in this family of tubular interstitial diseases, a disordered medullary architecture is characteristic, which might impair the efficient medullary recycling of potassium and thereby decouple the loop contribution to the excretion of an acute potassium load.

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Chapter 8

Cell Models of Potassium Transport in the Renal Tubule

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I. INTRODUCTION: ELEMENTS OF POTASSIUM TRANSPORT IN RENAL TUBULE CELLS

Potassium balance depends critically upon the kidney's ability to adjust potassium reabsorption and potassium secretion across specific segments of the nephron to the coordinated action of a broad spectrum of regulatory mechanisms. The regulatory potential of the kidney is impressive: Potassium excretion can be effectively matched to intake over a hundredfold range, yet only minor fluctuations in plasma potassium levels occur (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982).

In addition to the task of safeguarding potassium balance by maintaining a wide range of vectorial potassium transport rates across the renal tubules, epithelial cells of the kidney face an additional problem. To prevent compromising key cell functions such as that of several enzymes, acid–base homeostasis, and cell volume (Ussing, 1960), tubule cells must maintain their internal potassium concentration within narrow limits: Even dramatic changes in transepithelial potassium and sodium movement must be tolerated without disturbances of cell potassium concentration. This is achieved by complex interactions of potassium and sodium transport across the two limiting barriers of the renal epithelium, the apical and basolateral cell membranes. The problem of coordination and regulation of homocellular and transcellular transport of sodium and potassium in epithelia has recently been the focus of several incisive discussions by Schultz (1981, 1982).

At least four modes of potassium transport have been identified in tubule cells. They are ultimately responsible both for variable transepithelial net potassium

transport and for maintaining cell potassium at optimal levels. The following transport mechanisms have been identified:

1. The active, ATPase-dependent Na–K exchange pump in the basolateral cell membrane (Jorgensen, 1980; Mandel, 1985; DeWeer, 1985). It is responsible for the high potassium ion activity in tubule cells.
2. The passive leak conductance of potassium. Its distribution to the apical and basolateral cell membranes determines how much potassium recycles across the basolateral cell membrane or, alternatively, enters the tubule lumen. The high potassium conductance in the apical membrane of specialized tubule cells is a key factor in facilitating potassium secretion into the tubule lumen (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982).
3. The cotransport system of sodium, chloride, and potassium. It provides a mechanism for obligatory coupling of potassium to sodium chloride movement across the apical cell membrane of the cells of the diluting segment (Wright and Giebisch, 1985; Greger, 1985; Oberleithner *et al.*, 1982a).
4. Electroneutral potassium chloride cotransport may participate in the movement of potassium ions across the apical and basolateral cell membrane of some tubule cells (Greger, 1985; Ellison *et al.*, 1985).

All of these transport operations acting on potassium ions are also found in nonpolarized cells (Ussing, 1960). It is, however, the unique segregation of these transport mechanisms to the two limiting barriers, the apical and basolateral cell membranes, that allows control and regulation of *both* cell potassium homeostasis and transepithelial potassium movement.

II. BASIC CELL MODEL OF EPITHELIAL POTASSIUM TRANSPORT IN THE RENAL TUBULE

A. The Simple Two-Membrane Model of Epithelial Transport

Current concepts of the mode of operation of sodium-transporting epithelia such as the renal tubule are based on the double-membrane model originally proposed by Koefoed-Johnsen and Ussing (1958). Figure 1 summarizes its essential features and those modifications that account for its ability to explain net translocation of potassium ions.

Koefoed-Johnsen and Ussing (1958) suggested (1) that the apical cell membrane of sodium-transporting epithelia such as the frog skin is highly and selectively permeable to sodium ions (but not to potassium), (2) that the basolateral cell membrane is highly potassium selective (but not permeable to sodium ions), and (3) that the only *active* transport operation is the ATP-driven sodium–

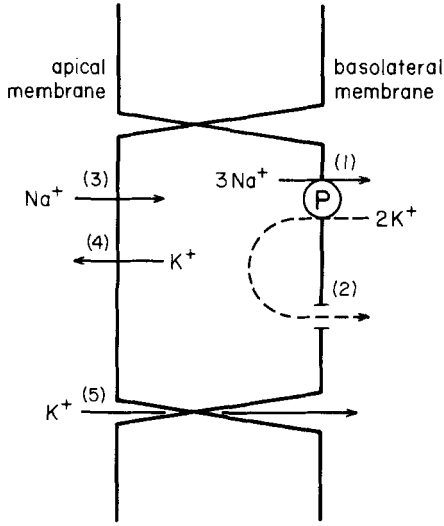

FIG. 1. Cell model of epithelial sodium and potassium transport. Note mechanisms of sodium and potassium movement across (1) the basolateral and (2) apical cell membrane and (3) across the intercellular shunt pathway. For details of transport operations 1–5, see text.

potassium exchange pump in the basolateral cell membrane. This distribution of passive sodium and potassium conductances and the unique localization of active Na–K exchange not only accounts for the high cell potassium and low cell sodium, but also for transepithelial movement of sodium from the apical to the basolateral solution.

This cell model is also applicable to the renal tubule. Provided some modifications are included, potassium secretion or reabsorption will occur. In the following, we shall consider these modifications of the transport operations that convert the "simple" original transport model of Koefoed-Johnsen and Ussing into the more complex cell models that endow tubule cells with the capacity of net potassium transport (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982).

Similar to frog skin cells, all tubule cells are characterized by active sodium–potassium exchange in the *basolateral* cell membrane. Also, potassium ions leak back from cells into the peritubular fluid. Thus, these transport properties, shown in Fig. 1 as operations 1 and 2, are common to both the original Koefoed-Johnsen–Ussing model and all renal tubule cell models (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982). However, it is the *apical* cell membrane of tubule cells that has developed special and additional transport functions. These concern the mode of sodium and potassium translocation. Rele-

vant aspects of these transport operations in the apical cell membrane of tubule cells will now be considered.

B. Sodium Movement

Sodium entry across the apical cell membrane (operation 3 in Fig. 1) is not limited to electrodiffusion as in the original frog skin model. In renal tubule cells, sodium entry may proceed by several mechanisms (Giebisch and Aronson, 1986; Burg, 1985). In the proximal tubule, cotransport with organic substances, or with other ions (Burg, 1985; Kinne *et al.*, 1975), and sodium–hydrogen exchange (Aronson, 1983; Murer *et al.*, 1976) are the main transport pathways. Activation of these transfer processes may depolarize the apical cell membrane (Frömter, 1982), but several sodium translocating processes are also electrically neutral. Apical Na–H exchange is a representative example (Aronson, 1983; Murer *et al.*, 1976).

In the mammalian thick ascending limb of Henle (Greger, 1985; Hebert and Andreoli, 1984) and in the diluting segment of amphibia (Oberleithner *et al.*, 1982a; Stanton and Giebisch, 1982), sodium reabsorption is coupled to the movement of chloride and, importantly, also to that of potassium. This cotransport is electrically neutral and plays a role in potassium reabsorption.

In the late distal tubule (initial collecting tubule) and the cortical collecting tubule, sodium entry occurs by electrodiffusion, similar to the mode of sodium transport across the apical cell membrane in the frog skin. This mode of sodium transport at the site of potassium secretion is significant. It accounts for asymmetrical cell polarization (relative depolarization of the apical membrane) and favors passive diffusive movement of potassium ions from cytoplasm to lumen (Wright and Giebisch, 1985; Giebisch, 1971; Koeppen *et al.*, 1983; Koeppen and Giebisch, 1985; O'Neil and Sansom, 1984a,b).

It is noteworthy that, ultimately, all mechanisms of sodium movement across the apical cell membrane are passive and driven by the sodium (and chloride) concentration gradient between lumen and cell fluid. The sodium concentration gradient across the apical cell membrane is generated by the active sodium–potassium exchange pump in the basolateral cell membrane.

C. Potassium Movement

Potassium ions traverse the apical cell membrane by several mechanisms (see Fig. 1, operation 4). Potassium movement from cell to lumen, accounting for potassium secretion, proceeds by diffusion and, accordingly, depends on the magnitude of the electrochemical potential gradient (driving force) of potassium and the potassium conductance. This type of potassium movement across the

apical cell membrane is the main pathway for potassium secretion across the distal tubule and the cortical collecting tubule (Wright and Giebisch, 1985; Giebisch, 1971; Koeppen *et al.*, 1983; Koeppen and Giebisch, 1985; O'Neil and Sansom, 1984a,b).

It is possible that an additional element of potassium secretion from cell to lumen involves passive, electroneutral potassium chloride cotransport (Ellison *et al.*, 1985). Such a mode of potassium movement may also account for passive potassium exit across the basolateral cell membrane of cells of the thick ascending limb of Henle's loop (Greger, 1985; Greger and Schlatter, 1983) and the diluting segment of *Amphiuma* (Guggino, 1985).

Potassium reabsorption from lumen to cell may involve also active uptake across the apical cell membrane (modified operation 3 in Fig. 1) (Wright and Giebisch, 1985; Giebisch, 1971). It is unknown whether this type of movement, not shown in Fig. 1, involves coupling to other ions.

Finally, passive movement of potassium ions between cells (operation 5 in Fig. 1) may also occur. This mode of transepithelial potassium movement probably plays a more important role in potassium reabsorption than in potassium secretion. Provided the transepithelial potential difference is lumen positive (late proximal tubule and thick ascending limb and diluting segment) and the potassium conductance of the paracellular pathway is high, significant potassium reabsorption may be mediated by this transport mechanism.

III. CONTROL OF CELL POTASSIUM

Renal tubule cells uniformly maintain potassium ion activity levels above electrochemical equilibrium. These high potassium levels are achieved by a balance between active uptake from the peritubular fluid and passive leakage from cell to extracellular fluid. Potassium ions are transferred into tubule cells by primary active ATP-driven Na^+—K^+ exchange in the basolateral cell membrane (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982). Tubule cells in the thick ascending limb of Henle's loop (Greger *et al.*, 1984) and in the cells of the amphibian diluting segment (Oberleithner *et al.*, 1982b, 1983a) also transport potassium ions actively across the luminal cell membrane, but inhibition of such (secondary) active potassium transport does not reduce cell potassium activity as long as the basolateral Na^+–K^+ pump continues to operate. Accordingly, active potassium uptake in the basolateral cell membrane of these tubule cells compensates for the lack of potassium uptake across the apical cell membrane so that high cell potassium levels are maintained even after potassium transport across the apical membrane has been effectively blocked (Oberleithner, 1982b, 1983a).

A. Active Sodium–Potassium Exchange

The active nature of potassium uptake into kidney cells is demonstrated by measurements of cell potassium activities, both in amphibian (Oberleithner *et al.*, 1982b, 1983a; Lang *et al.*, 1983; Messner *et al.*, 1985c; Teulon and Anagnostopoulos, 1982a; Sackin and Boulpaep, 1981; Kubota *et al.*, 1983a,b) and in mammalian (Greger *et al.*, 1984; Edelman *et al.*, 1978; Cemerikic *et al.*, 1982; Biagi *et al.*, 1981a) tubule cells. All such measurements indicate potassium activities above those to be expected from the magnitude of the negative cell potential. Thus, intracellular potassium is maintained at higher activities than predicted for passive diffusion. This generates a driving force for potassium efflux from the cell of the order of some 20–30 mV that requires balancing by active potassium transport from the extracellular fluid into the cell. Hence, intracellular potassium levels are set by a pump-leak system where passive potassium efflux is balanced by active, pump-driven influx.

The sensitivity of potassium activity levels to pump inhibitors underscores the importance of active potassium transport. Figure 2 shows results from experiments in which the effects of ouabain on cell potential and potassium activities were explored (Kubota *et al.*, 1983a). The relationship between membrane potential and cell K activity at lower ouabain concentrations indicates still some active potassium uptake, since the cell potassium activity exceeds that expected for passive distribution. At the higher ouabain concentrations, though, the membrane potential approaches the calculated Nernst potential for potassium. This would be expected if, with increasing pump inhibition, passive membrane processes were now sufficient to account for potassium distribution across tubular cell membranes.

B. Electrogenic Nature of Sodium–Potassium Exchange

Several lines of evidence suggest that the exchange rate of sodium for potassium is unequal. It is likely that an exchange ratio of three sodium ions for two potassium ions is the most common mode of operation (Nielsen, 1979; Mandel, 1985; DeWeer, 1985; Avison *et al.*, 1987), although deviations from this ratio have been reported (Sackin and Boulpaep, 1983; Sansom and O'Neil, 1985).

The contribution of electrogenic cation exchange to the steady-state voltage across the basolateral membrane of tubule cells is normally small, but activation of electrogenic Na^+–K^+ pumping, either by raising extracellular potassium from low values or by rewarming tubules, following a period of pump depression by cooling, leads to transient hyperpolarization of the basal cell membrane. Potential levels have been observed that are distinctly above those expected if passive diffusion of potassium were the sole mechanism controlling the mem-

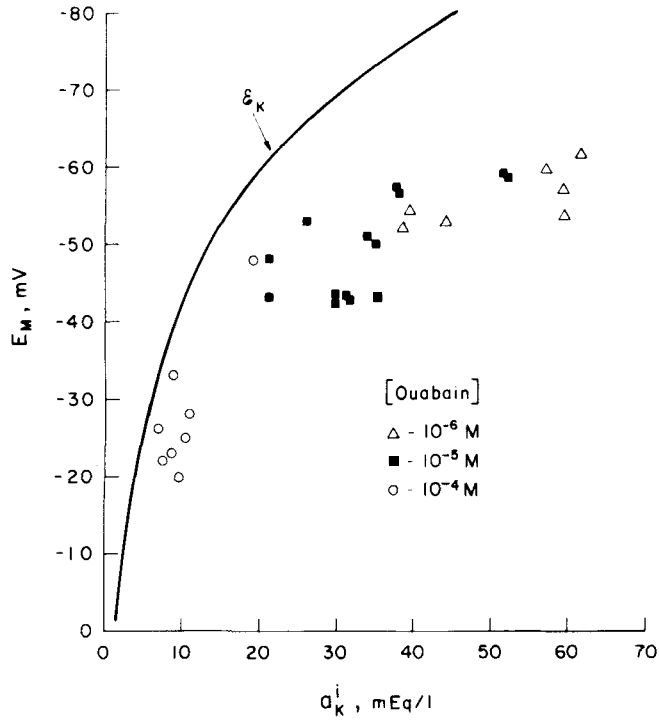

FIG. 2. Relationship between cell membrane potential and the intracellular potassium activity (a_K^i) at different concentrations of ouabain. The solid line represents the Nernst equilibrium potential for K. [From Kubota *et al.* (1983a).]

brane potential (Proverbio and Whittembury, 1975; Sackin and Boulpaep, 1981, 1983; Biagi *et al.*, 1981b).

Figure 3 shows an example of electrogenic sodium–potassium exchange in a proximal tubule cell of a perfused rabbit tubule *in vitro* (Biagi *et al.*, 1981b). Both conventional and ion-sensitive electrodes were used simultaneously to measure cell potentials and potassium activities. Tubules were first exposed to a low potassium bath, and subsequently, potassium was raised. This concentration step resulted in a rapid hyperpolarization of the basolateral cell membrane. Importantly, during this recovery phase the membrane potential (cell negative) significantly exceeded the measured potassium equilibrium potential. This relationship between membrane potential and potassium equilibrium potential demonstrates the operation of electrogenic sodium–potassium exchange in tubule cells. Similar results were also obtained in amphibian proximal tubules (Sackin and Boulpaep, 1981, 1983). Subsequently we shall discuss observations made in

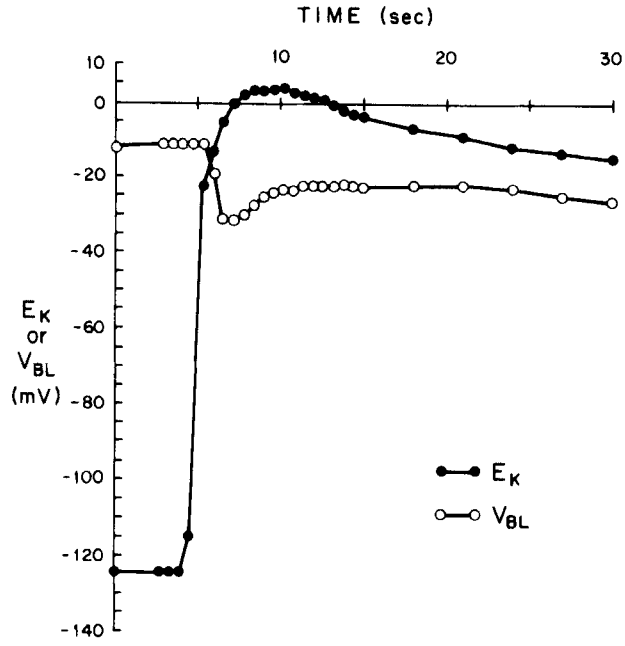

FIG. 3. Transient relationship between V_{BL}, the basolateral membrane potential, and E_K, the potassium equilibrium potential, in perfused rabbit straight proximal tubule. Recovery from low bath potassium is shown. The rapid hyperpolarization following addition of potassium to the bath is due to reactivation of electrogenic sodium–potassium exchange. [From Biagi *et al.* (1981a).]

single cells of the perfused cortical collecting tubule, demonstrating that during the activation of the sodium–potassium pump by mineralocorticoids, the basolateral cell potential may also reach levels that exceed the potassium equilibrium potential (Koeppen *et al.*, 1983; Koeppen and Giebisch, 1984; Sansom and O'Neil, 1985). Again, this may be taken as evidence in support of an electrogenic mode of operation of the sodium–potassium pump.

C. Factors Controlling Cell Potassium in Tubule Cells

Several conditions are known in which cell potassium activities are reduced. Besides pump inhibition by ouabain (Teulon and Anagnostopoulos, 1982a; Sackin and Boulpaep, 1981; Biagi *et al.*, 1981a; Kubota *et al.*, 1983a; Planelles and Anagnostopoulos, 1982) and cold (Sackin and Boulpaep, 1981, 1983), metabolic acidosis (Cemerikic *et al.*, 1982; Kubota *et al.*, 1983b) and dietary potassium depletion (Cemerikic *et al.*, 1982) as well as acute reduction of per-

itubular potassium levels (Kubota *et al.*, 1983a; Matsumura *et al.*, 1984b) lower cell potassium levels in proximal tubule cells. Diminished active uptake in the presence of continued loss of potassium ions by diffusion is responsible for such reductions in cell potassium.

Adaptation to high ambient potassium, in contrast, raises cell potassium activities in cells lining the diluting segment of the *Amphiuma* kidney (Oberleithner *et al.*, 1983b). Metabolic alkalosis also increases the cell potassium activity in proximal tubule cells of *Necturus* (Kubota *et al.*, 1983b). Again, it is most likely that such changes of cell potassium are mediated, in part at least, by stimulation of active basolateral sodium–potassium exchange.

D. Effects of Changes in Extracellular Potassium on Tubular Transport Processes

A large number of apical transport processes of tubule cells depend upon adequate operation of active, basolateral sodium–potassium exchange. It is this active, potassium-dependent sodium extrusion across the basolateral cell membrane that effectively lowers cell sodium activity, providing the energy for "secondary" active transport processes that depend upon the sodium concentration gradient across the luminal cell membrane (Giebisch and Aronson, 1986; Aronson, 1981). In addition, active sodium–potassium exchange also determines the rate of sodium–calcium exchange across the basolateral cell membrane of renal tubule cells (Windhager and Taylor, 1983). The activity of the basolateral sodium–potassium exchange pump also accounts for fluid transport across all segments of the nephron by providing the osmotic driving force responsible for effective solute–solvent coupling that links transepithelial sodium to fluid movement (Giebisch and Aronson, 1986; Burg, 1985).

Since the activity of the basolateral sodium–potassium pump determines cell sodium activity in tubule cells, impairment of its turnover by reduction of basolateral potassium affects, *pari passu*, the sum of those co- and countertransport processes that depend critically upon the sodium concentration gradient between lumen and cytoplasm. Figure 4 provides an overview of those transport operations that depend upon the electrochemical sodium gradient from extracellular fluid to cytoplasm. To the extent that the basolateral sodium–potassium exchange is potassium dependent, the concentration of potassium in the peritubular environment determines the activity of the sodium extrusion mechanism.

The importance of basolateral sodium–potassium exchange for transepithelial fluid and sodium transport was carefully investigated in experiments in which the effects of luminal and basolateral changes in potassium concentration were explored. Figure 5 shows a representative example in the isolated, perfused

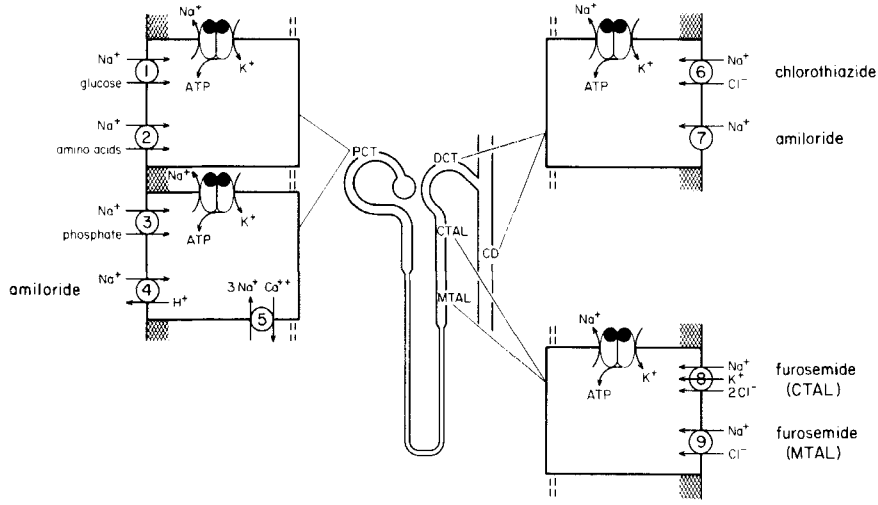

FIG. 4. Summary of potassium-dependent transport processes along the nephron. Sodium–potassium exchange in the basolateral cell membrane of tubule cells is an active process and coupled to ATP hydrolysis. This pump activity generates a steep electrochemical sodium gradient from extracellular fluid to cytoplasm. Accordingly, active sodium–potassium exchange is responsible for energizing and for providing the driving force for secondary co- and countertransport processes in the apical and basolateral cell membrane. [Modified from Jorgensen (1980).]

proximal tubule of the rabbit (Cardinal and Duchesneau, 1978). In these experiments the effects of changes in extracellular potassium upon fluid absorption depend critically upon the site where potassium ions are reduced. Whereas changes in luminal potassium have no marked effect upon proximal fluid absorption, a sharp inhibition of fluid transport follows reduction in peritubular potassium concentration. Fluid transport is completely inhibited in the absence of peritubular potassium (see also Kokko *et al.*, 1971; Schafer *et al.*, 1974). When the concentration of potassium in the peritubular environment is progressively increased, fluid transport is stimulated. It reaches saturation at potassium levels of about 2.5 mEq/liter. There is no evidence in this tubule preparation that potassium-independent (and ouabain-insensitive) sodium extrusion (Whittembury and Proverbio, 1970) participates in transepithelial sodium and fluid transport.

Sodium transport in the thick ascending limb of Henle is also sensitive to changes in plasma potassium, i.e., to changes in basolateral potassium concentration. Thus, attention has recently been drawn to the relationship between potassium concentration and sodium transport in dietary potassium depletion in which a gradual decline in plasma potassium could be achieved (Gutsche *et al.*, 1984). The key observation here concerns the partial collapse of the trans-

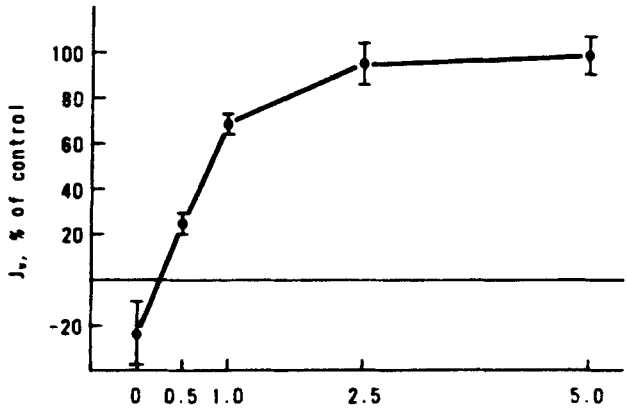

FIG. 5. Effect of changes in extracellular potassium concentration on fluid transport in the isolated proximal convoluted rabbit tubule. Results are expressed in percentage of control value. The luminal perfusion fluid always contained 5 mEq/liter of potassium. [From Cardinal and Duchesneau (1978).]

epithelial sodium concentration gradient in the early distal tubule following the development of potassium deprivation. Figure 6 summarizes the relationship between plasma potassium concentration and the reduction of the steady-state ("static head") chloride concentration gradient. It is apparent that the ability of the thick ascending limb to lower solute (i.e., sodium) concentration is increasingly impaired as the potassium concentration in the plasma falls. This functional impairment could be readily reversed by acute administration of potassium, which raises the peritubular potassium level. Again, restoration of luminal potassium delivery to the diluting segment was ineffective. The most reasonable explanation of these results is a rate-limiting effect of low peritubular potassium concentrations upon basolateral sodium–potassium pump activity.

Additional potassium-sensitive transport functions in renal tubules that depend critically upon the integrity of basolateral sodium–potassium exchange are volume regulation in mammalian proximal tubules (Grantham *et al.*, 1977; Dellasega and Grantham, 1973), operation of basolateral sodium–calcium exchange (Windhager and Taylor, 1983), and tubular bicarbonate transport (Burg and Green, 1977; Chantrelle *et al.*, 1982; Chan and Giebisch, 1981). A detailed discussion of these events is beyond the scope of this review.

E. Control of Basolateral Potassium Permeability

1. Potassium Permeability and Pump Activity

Cell potassium activity not only depends upon the integrity of active sodium–potassium exchange, but also upon the magnitude of the passive potassium leak

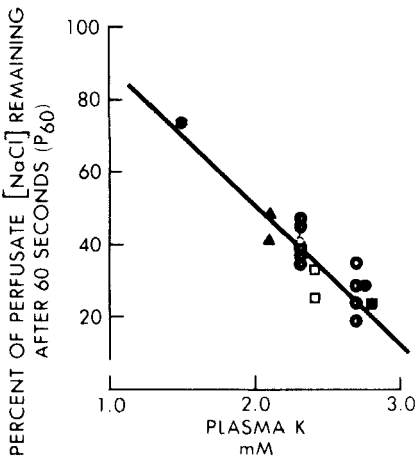

FIG. 6. Relationship between plasma potassium concentration in potassium-depleted rats and the percentage of the steady-state NaCl concentration remaining after a 60-sec stop-flow interval. Symbols represent individual measurements in different animals. [From Gutsche *et al.* (1984).]

out of cells. In the steady state, and particularly in those cells of the nephron that lack significant apical potassium pathways such as proximal tubule cells, these two processes—active potassium uptake by the pump and passive, dissipative leak of potassium ions out of the cell across the basolateral cell membrane—must operate at equal rates.

The situation following pump stimulation is of particular interest. Given a constant coupling ratio of sodium extrusion to potassium uptake and constant cell volume, activation of sodium extrusion across the peritubular cell membrane must be associated with an increase in the potassium leak (Schultz, 1981, 1982; Cohen and Giebisch, 1984; Lang *et al.*, 1986). Otherwise, dramatic changes in cell potassium content and cell volume would occur. Such increased exit of potassium ions with pump stimulation could, in principle, occur by two mechanisms. It would be mediated either by an increase in the driving force for potassium exit or, alternatively, by an increase in basolateral potassium conductance. A large body of evidence is now available which supports the view that the potassium activity in renal tubule cells does not significantly change with alterations in pump activity. It is, rather, by varying the magnitude of the passive leak conductance to potassium that cells of the renal tubule maintain cell potassium activity and cell volume within narrow limits, despite large changes in the rate of active, basolateral sodium extrusion.

We have recently addressed this problem in a series of transport and electrophysiological studies on proximal tubule cells in the isolated *Necturus* kidney (Kubota *et al.*, 1983a; Matsumura, 1984b). Two observations are relevant. First,

TABLE I
EFFECTS OF INHIBITION OF SODIUM TRANSPORT ON BASOLATERAL POTASSIUM TRANSFERENCE NUMBER[a]

	V_{bl} (mV)	a_K^i (m*M*)	t_K
Control	59 ± 2 (17)[b]	67 ± 2 (17)	0.38 ± 0.03 (15)
Low K^+ perfusion	39 ± 2 (12)	49 ± 3 (12)	0.09 ± 0.01 (21)
	$P < 0.001$	$P < 0.001$	$P < 0.001$
Control	54 ± 1 (8)	77 ± 5 (8)	0.32 ± 0.03 (12)
10^{-4} *M* ouabain	14 ± 2 (8)	26 ± 3 (8)	0.19 ± 0.02 (9)
	$P < 0.001$	$P < 0.001$	$P < 0.01$
Control	61 ± 1 (8)	—	0.23 ± 0.03 (8)
Low luminal Na^+	53 ± 3 (6)	—	0.11 ± 0.03 (6)
	$P < 0.05$		$P < 0.02$
Control	68 ± 1 (28)	—	0.38 ± 0.02 (28)
Low basolateral Na^+	47 ± 1 (8)	—	0.20 ± 0.02 (8)
	$P < 0.01$		$P < 0.001$

[a] V_{bl}, Basolateral membrane potential; a_K^i, intracellular potassium activity; t_K, potassium transference number. [From Matsumura *et al.* (1984a).]

[b] Number of observations is given in parenthesis.

potassium activities were shown to remain constant despite sharp reduction in net sodium transport across the proximal tubule (Kubota *et al.*, 1983a). This was achieved in the absence of significant changes of the electrochemical gradient of potassium across the basolateral cell membrane. Hence, changes in the electrochemical driving force were not involved in the modulation of basolateral potassium exit.

Second, in these studies we have also evaluated the relationship between basolateral potassium conductance and net sodium transport rate (Matsumura *et al.*, 1984b). Relevant data are summarized in Table I. Sodium transport was modified in several different ways, either by direct pump inhibition (ouabain), reduction in basolateral potassium concentration, low luminal sodium, or by reduction in basolateral sodium concentration. The latter maneuver has been shown to increase cell calcium and to inhibit entry of sodium into the cell. The effect of these manipulations upon the transference number of potassium was assessed by measuring the change in basolateral membrane potential induced by step changes in basolateral potassium concentration.[1]

[1] Transference numbers are calculated by the expression $tc = \Delta V_{bl}/58 \log(C_2/C_1)$where V_{bl} is the inital peak change in basolateral potential due to the concentration change of the test ion and C_1 and C_2, the two concentrations of ion C used (Matsumara *et al.*, 1984a)

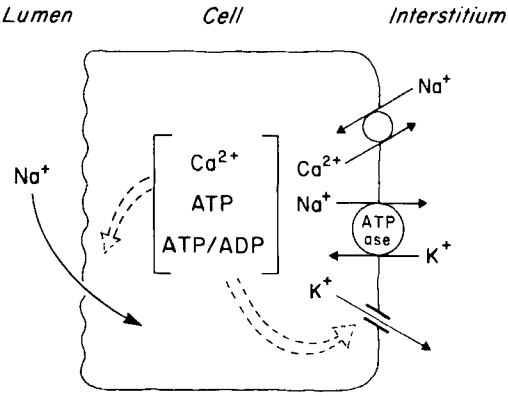

FIG. 7. Tubule cell model with several transport mechanisms that may be involved in the coupling of net sodium movement to the luminal sodium and the basolateral potassium permeability. Note presence of a potassium conductance, an active ATPase-driven sodium–potassium exchange, and a sodium–calcium exchange mechanism in the basolateral cell membrane. Cytoplasmic calcium may affect both apical sodium entry and the basolateral potassium conductance. [From Wright and Giebisch, (1985); redrawn from Cohen and Giebisch (1984).]

From inspection of Table I, we note that sodium transport inhibition is uniformly associated with a reduction of t_K, the transference number of potassium ions. Transport inhibition is accompanied by a reduction of the basolateral electrical potential difference. A similar tight relationship between sodium–potassium pump rate and basolateral potassium conductance has also been reported in the proximal tubule of the frog kidney (Lang *et al.*, 1983, 1986; Messner *et al.*, 1985c), the mammalian proximal tubule (Völkl and Greger, 1984), and in several other epithelia (Schultz, 1981, 1982; Lau *et al.*, 1984).

The cell mechanism by which coupling between sodium–potassium exchange and potassium conductance occurs is not fully understood, but some possibilities have recently been suggested. Figure 7 includes mechanisms that could regulate the relationship between basolateral pump rate and potassium conductance (Wright and Giebisch, 1985; Ussing, 1960; Schultz, 1981; Cohen and Giebisch, 1984). Shown is a cell model that incorporates relevant components of sodium and potassium transport. Sodium ions enter the cell along a favorable electrochemical gradient.[2] Two transport mechanisms are depicted in the basolateral

[2] As previously discussed (see Fig. 4), such entry of sodium ions may occur by a variety of mechanisms, including cotransport mechanisms that transfer positive charge into the cell (Giebisch and Aronson, 1986; Burg, 1985; Frömter, 1982). Potassium ions are the most permeant ion species in the basolateral cell membrane. Hence, they provide an important pathway for transcellular current flow and are thus crucially involved in the activity of rheogenic, sodium-coupled cotransport mechanisms in the apical cell membrane.

cell membrane: ATPas-dependent sodium–potassium exchange, and a sodium entry pathway that is linked to the extrusion of calcium ions by an exchange mechanism (Windhager and Taylor, 1983). It can be shown that the rate of calcium extrusion is determined by the magnitude of the electrochemical gradient for sodium across the basolateral membrane. Accordingly, intracellular calcium activity changes in the same direction as intracellular sodium activity: A reduction in active, basolateral sodium extrusion increases cytosolic calcium activity (Windhager and Taylor, 1983; Lorenzen *et al.*, 1984). It has been suggested that these changes in cell calcium activity regulate the rate of sodium entry across the apical cell membrane and thus couple peritubular pump rate to apical sodium transport (Windhager and Taylor, 1983).

The basolateral potassium permeability may also depend on the level of cell calcium. This hypothesis is based on the recognition that, similar to nonrenal cells, tubule cells have a calcium-activated potassium conductance. The latter is inhibited by barium ions and activated by a number of maneuvers that have in common an increase in cell calcium levels (Gullans *et al.*, 1985; Chase, 1984).

The following events have been suggested to account for the coupling between basolateral pump rate and potassium conductance. Cell sodium activity might rise, at least temporarily, following increased entry of sodium ions across the apical cell membrane.[3] This event would decrease the sodium concentration gradient across the basolateral cell membrane and reduce the driving force for calcium extrusion. The rise in calcium ions could then initiate an increase in potassium conductance in the basolateral cell membrane (Cohen and Giebisch, 1984). The opposite should occur when luminal sodium entry is reduced (Lorentzen *et al.*, 1985).

Figure 7 shows alternative mechanisms of coupling active sodium extrusion to potassium conductance. One is the possibility that transport-related alterations in metabolism, such as changes in the ATP level, or the ATP/ADP ratio, change the sensitivity of the potassium conductance to calcium ions. Finally, it should also be considered that changes in the phosphorylation of the potassium channel could coincide with changes in pump activity and modulate the potassium conductance. Precedence for such a mechanism of control of potassium channels is available both in nonexcitable and in excitable tissues (see Cohen and Giebisch, 1984; Schwartz and Passow, 1983).

The "calcium-signal" hypothesis cannot explain all observations. Several instances are known in which an increase of cell calcium activity is associated with a fall in potassium conductance and not, as expected, with augmentation of the potassium leak. Relevant examples are the reduction of pump activity by ouabain (Messner *et al.*, 1985a) or by low peritubular potassium (Kubota *et al.*,

[3] Stimulation of sodium entry can be induced by raising the luminal sodium concentration, by adding organic substrates to the lumen, or by stimulating sodium–hydrogen exchange.

1983a). Both situations are associated with a reduction of sodium–potassium exchange and a reduction of potassium conductance. However, a sharp increase in cell calcium activity has been observed after basolateral pump inhibition by ouabain (Messner *et al.*, 1985a).

2. Potassium Permeability and Membrane Potential

Several studies show a tight relationship between basolateral potassium conductance and the magnitude of the basolateral membrane potential (Matsumura *et al.*, 1984b; Lang *et al.*, 1986). A decrease in basolateral potassium conductance follows manipulations that lower the pump rate and the basolateral cell potential. It is also observed in conditions in which the basolateral potential depolarizes following addition of substrate to the lumen (Messner *et al.*, 1985b). Finally, a fall in potassium conductance also occurs after appropriate current injection into tubule cells that depolarizes the basolateral membrane potential (Messner *et al.*, 1985b). The potential dependence of the basolateral potassium conductance can also be observed in single potassium channels found in the basolateral membrane patches of *Necturus* proximal tubule cells (Kawahara *et al.*, 1985a). Finally, Lang and his associates have shown that, in addition to membrane depolarization, a reduction of cell potassium activities per se may lower the potassium conductance (Lang *et al.*, 1985, 1986).

3. Potassium Permeability and the Effects of pH, Calcium, and Barium

Another property of the peritubular potassium conductance is its pH dependence. In general, acidifying the external medium reduces the potassium leak of tubule cells; alkalinization has the opposite effect. Figure 8 shows results of experiments on the perfused *Necturus* kidney. External pH was altered by changing bicarbonate at constant P_{CO_2} (Kubota *et al.*, 1983b). We note that between pH 6.4 and 8.15, the relationship between basolateral cell potential difference and pH was sigmoidal, with the highest sensitivity in the range of 6.8–7.6. Cell potassium activity measurements are also included in the lower pannel of Fig. 8. It is apparent that a significant fall in cell potassium occurred with more acid pH in the external medium. Could the fall in cell potassium activity have been responsible for the depolarization of the basolateral membrane potential? The reduction of the basolateral membrane potential, from 67 to 20 mV, is consistent with a decrease in cell potassium activity, but the magnitude of the measured depolarization greatly exceeds that to be expected from the fall in cell potassium. This can be shown by comparison of the Nernst potential calculated for the measured potassium activity difference with the observed depolarization of the membrane potential. Whereas the latter changed by 47 mV, the potential change attributable to the fall in cell potassium was only 8 mV. This discrepancy is best

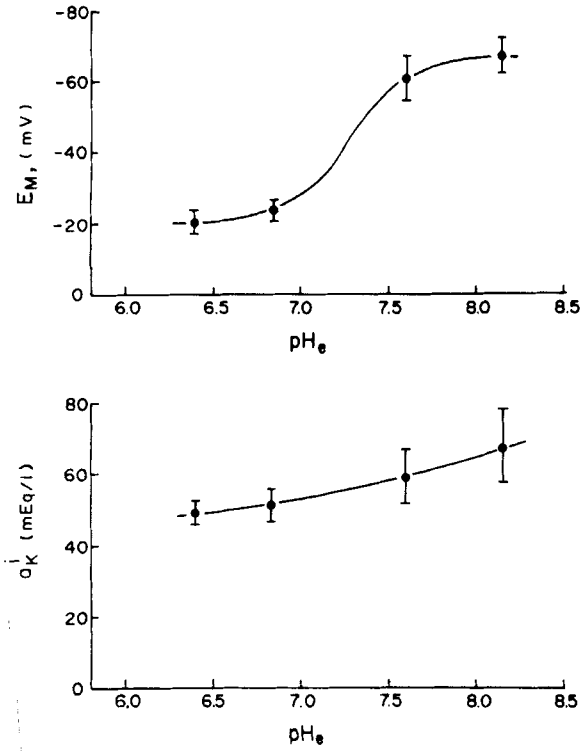

FIG. 8. Relationship between basolateral membrane potential (E_m) and the intracellular potassium activity (a_K^i) to the pH of the extracellular fluid. Steady-state values obtained after 1-hr perfusion are summarized. Results were obtained on proximal tubule cells in the perfused *Necturus* kidney. [From Kubota *et al.* (1983a).]

explained by a reduction in basolateral potassium conductance following acidification of the extracellular and cellular fluid (Kubota *et al.*, 1983b).

The response of the basolateral membrane potential in rabbit proximal straight tubule cells to changes in extracellular pH also supports the view that the potassium permeability is decreased during acidosis (Biagi *et al.*, (1981b). Studies on the apical cell membrane of the diluting segment in *Amphiuma* and the rabbit cortical collecting tubule have further supported the view that acidification of the cytoplasm and the luminal fluid lowers the potassium permeability (Stanton *et al.*, 1982; Oberleithner *et al.*, 1985a; O'Neil, 1983). The possible role of such alterations in potassium permeability in the regulation of potassium secretion across renal tubules will be discussed later.

Additional experimental evidence is available that demonstrates a fall of the basolateral potassium permeability of proximal tubule cells after exposure to

barium ions, a well-known inhibitor of potassium channels (Biagi *et al.*, 1981a; Bello-Reuss, 1982; Matsumura *et al.*, 1984a; Planelles *et al.*, 1981; Hunter *et al.*, 1984). Similar to other epithelia (see Chase, 1984), the basolateral potassium permeability of proximal tubules rises with increasing cell calcium (Gullans *et al.*, 1985).

4. Potassium Permeability and Cell Volume

An additional factor that may affect the potassium permeability of kidney cells is a change in cell volume. An increase in cell volume leads, in general, to an increase in the potassium conductance of epithelial cells (Chase, 1984), and kidney cells share this behavior (Grantham *et al.*, 1977; Dellesega and Grantham, 1973; Whittembury *et al.*, 1978). An increase in cell calcium may trigger this activation of basolateral potassium conductance (Chase, 1984).

Schultz and his associates have suggested that the delayed increase in basolateral potassium conductance that follows stimulation of sodium transport in the small intestine is related to the volume increase that follows the enhancement of substrate-dependent sodium entry into these epithelial cells (Lau *et al.*, 1984). This view is supported by observations that the change in potassium permeability could be mimicked by relatively small increments in cell volume, induced by exposing these epithelial cells to a 12% hypotonic medium. Guggino and Lopes (1985) have observed that exposure of proximal tubule cells of *Necturus* to similarly hypotonic media also increases the basolateral potassium conductance. It is thus tempting to speculate that enhanced luminal sodium entry and stimulation of basolateral sodium transport, if followed by modest cell swelling, perhaps signal a change in cytosolic calcium activity and activation of a basolateral potassium conductance.

5. Electrical Coupling between Basolateral and Apical Cell Membranes

The important relationship between sodium transport and basolateral potassium conductance also plays a critical role in linking membrane events between the apical and basolateral cell membranes. In leaky epithelia, such as the proximal tubule, stimulation of sodium transport, for instance, by addition of organic substrates to the lumen, results in cell depolarization because such cotransport carries positive current into the cell (Samarzija *et al.*, 1982). Although epithelial sodium transport increases, this also tends to curtail further sodium entry because the depolarization of the apical cell membrane reduces the electrochemical driving force for sodium transport.

The increase in basolateral potassium conductance following basolateral pump stimulation may, however, provide a feedback loop that acts to support continuation of sodium transport at accelerated rates. Critical for such sustained transport

stimulation is the increase in basolateral potassium conductance and the hyperpolarization of the basolateral cell membrane. Such secondary hyperpolarization has been observed in proximal tubule cells (Lapointe *et al.*, 1984; Messner *et al.*, 1985b) and in intestinal epithelium (Lau *et al.*, 1984). This increase in basolateral cell polarization renders the cell more negative and generates current flow through the intercellular low-resistance shunt pathway. As a consequence of such intraepithelial current flow (Boulpaep, 1979), the apical cell membrane repolarizes and the driving force for sodium entry increases. Evidence supporting such a sequence of events has been obtained in both mammalian and amphibian proximal tubule cells (Lapointe *et al.*, 1984; Messner *et al.*, 1985b).

In conclusion, it appears that the coupling between sodium transport and basolateral potassium conductance plays a dual role. First, the linkage between the passive potassium leak and the active sodium–potassium pump serves to prevent fluctuations of cell potassium activity and content during variations in pump activity. In addition, pump-dependent alterations in potassium conductance tend to stabilize and restore the apical membrane potential following transport stimulation. This assures optimal electrochemical sodium gradients during stimulation of transport. Not all aspects of the mechanisms that affect the basolateral potassium permeability are understood. The control of potassium permeability is complex and, as pointed out, subject to regulation by several factors.

F. Single Potassium Channels in Tubule Cells

Since the introduction of the patch-clamp technique of single-channel recording (Hamill *et al.*, 1981), progress has been made in the definition of potassium conductive pathways in apical and basolateral membranes of the tubular epithelium.

Figure 9 provides a schematic summary of the technique and its most frequent application to tubule cell membranes (Hamill *et al.*, 1981). In essence, the method consists of electrically isolating a small membrane patch, applying command voltages across the membrane, and exposing it to solutions of known composition. If channels are present, they manifest themselves by spontaneous openings and closings, i.e., current fluctuations. These depend on the voltage across the membrane patch and on the ion concentrations of the solutions bathing the inside and outside of the membrane patch and the inherent properties of the channels.

The details of the patch-clamp method are shown in Fig. 9 (Hamill *et al.*, 1981). The first panel shows the position of the pipette tip in close contact with the cell surface. The second panel demonstrates formation of a high-resistance seal (gigaohm range) by applying modest suction. Recordings of single po-

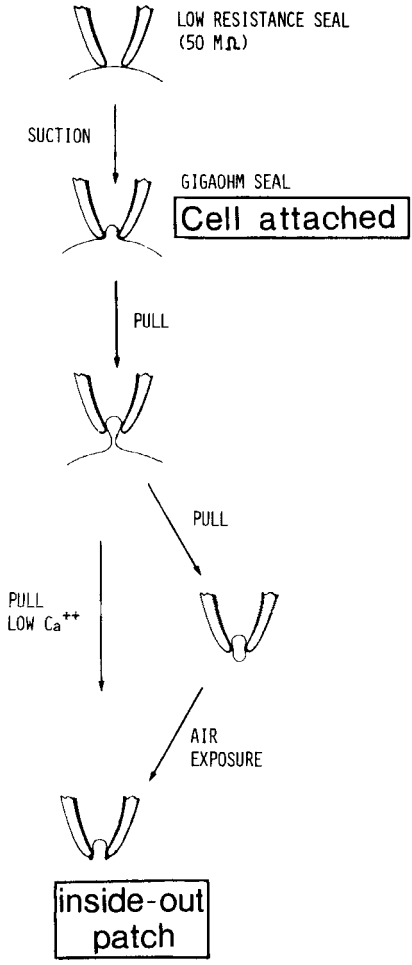

FIG. 9. Schematic representation of steps that lead to recording of single-channel activity in cell-attached and inside-out patches of tubule cell membranes. Note the formation of a high-resistance seal between pipette and cell membrane (gigaohm seal) and the subsequent formation of an inside-out membrane patch by pulling the patch off the cell and by air exposure. [Modified from Hammill *et al.* (1981).]

tassium channels in this "cell-attached" mode have been obtained from the basolateral and the apical cell membrane of single tubule cells (Hunter *et al.*, 1984; Koeppen *et al.*, 1984; Gögelein and Greger, 1984; Kawahara *et al.*, 1985a; Palmer, 1986). Figure 9 also demonstrates the formation of "inside-out patches." This is a cell-free recording configuration which is obtained by first pulling the patch off the cell and then briefly exposing it to air. This method has been applied successfully to the study of single channel currents in tubule cells.

Two types of preparations have been particularly useful in studies of ion channels in renal tissue. The first involves the use of the single isolated tubule preparations. Recordings have been made both from the apical and basolateral cell membranes. The most difficult step is that of adequately exposing the luminal and basolateral aspects of the tubule cells for recording. Concerning apical recordings, tubules have either been opened by ripping the tubule lengthwise with a fine needle (Hunter *et al.*, 1984; Koeppen *et al.*, 1984; Palmer and Frindt, 1987; Kawahara *et al.*, 1985a) or by inserting the recording patch pipette longitudinally into the lumen (Gögelein and Greger, 1984). The peritubular cell membrane can be approached from either the cut end of the isolated tubule (Gögelein and Greger, 1984) or, alternatively, the basal cell membrane can be exposed by careful removal of the connective tissue layer, including the basal membrane. A second approach has been the use of flat sheets of renal tubule cells grown in cultures (Guggino *et al.*, 1985a,b).

Potassium-selective channels have been identified both in the luminal and the basolateral cell membrane of proximal tubule cells (Kawahara *et al.*, 1985a; Gögelein *et al.*, 1984) and in apical cell membranes of cells lining the diluting segment of *Amphiuma* (Kawahara *et al.*, 1986) and the mammalian cortical collecting tubule (Hunter *et al.*, 1984; Koeppen *et al.*, 1984; Palmer, 1986). Sodium channels have also been recorded in patches from the apical cell membranes of collecting tubules (Palmer and Frindt, 1987a,b). In addition, potassium channels have also been identified in the luminal membrane of cultured tubule cells (Guggino *et al.*, 1985a,b).

Figure 10 shows recordings of two potassium channels obtained from proximal tubule cells of *Necturus* (Kawahara *et al.*, 1985a). Recordings from patches of the luminal membrane in the cell-attached and the excised mode (inside out) both show single-unit conductances. The channel is largely closed in the cell-attached mode, but in the inside-out mode two distinct channels are apparent.

A summary of the properties of single apical and basolateral channels of proximal tubule cells of *Necturus* is given in Table II. Of particular interest is the

TABLE II

SUMMARY OF PROPERTIES OF POTASSIUM CHANNELS IN THE LUMINAL AND BASAL MEMBRANE OF PROXIMAL TUBULE CELLS OF NECTURUS[a]

	Luminal	Basal
Single-channel conductance	25 pS	50 pS
Selectivity coefficient	>25	≈20
Open probability increased with depolarization		Increased hyperpolarization
Ba^{2+} effect (cytoplasm)	+	− (K gradient dependent)

[a] From Kawahara, Hunter, and Giebisch (unpublished).

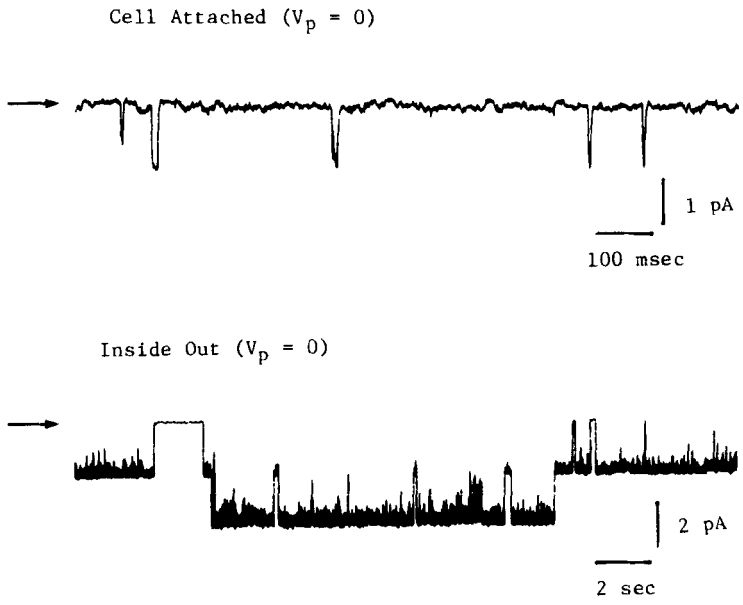

FIG. 10. Recording of single potassium channel activity in the luminal membrane of *Necturus* proximal tubule cells. Upper panel shows results obtained in the cell-attached recording mode; lower panel depicts activity in an excised membrane patch. Note presence of two distinct channels. [From Kawahara, Hunter, and Giebisch (1985a and unpublished).]

high potassium selectivity of both channels and their different behavior to voltage changes. Whereas the probability of the apical channel to open increases with depolarization, the opposite is true for the basolateral channel. Its tendency to open with hyperpolarization and to close with depolarization is of particular interest, since it is consistent with the behavior of macroscopic potassium currents in proximal tubule cells of *Necturus*. Thus, based on potential measurements following step changes in extracellular potassium concentration at different initial potential levels, a fall in potassium conductance is uniformly observed with cell depolarization. This behavior explains the tendency of tubule cells to shut off their basolateral potassium permeability (and to curtail loss of potassium ions) during cell depolarization (Matsumura *et al.*, 1984b; Messner *et al.*, 1985a; Lang *et al.*, 1985a, 1986).

IV. NET TRANSPORT OF POTASSIUM

In the following, we shall review what is presently known about the transport of potassium at several strategically important sites along the nephron. An effort will be made to analyze net potassium movement in terms of transport events at

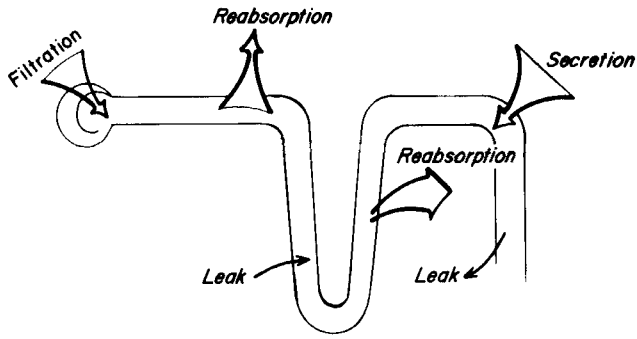

FIG. 11. Main sites of potassium transport along the nephron.

the level of single cell membranes. In addition, transport of potassium between epithelial cells, through the intercellular shunt pathway, will be considered.

Figure 11 provides an overview of the strategic sites of potassium transport along the nephron (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982). Virtually all of the potassium in plasma water is freely filtrable and hence available for filtration across the glomerular membrane. A large amount of potassium is reabsorbed along the proximal convoluted tubule. In addition, potassium ions are also reabsorbed along the loop of Henle, particularly across the thick ascending limb. By the time tubular fluid has reached the late distal tubule, a very large fraction of the filtered potassium has disappeared from the lumen. Depending on the metabolic situation, potassium is now added to the tubular fluid. It is in the late distal tubule (the initial collecting duct) and the cortical collecting duct where potassium secretion takes place, and it is this secretory transport operation that essentially determines the amount of potassium in the final urine.

The problem of potassium recycling, of particular relevance in the juxtamedullary nephron population, is analyzed in Chapter 7 by Jamison and Müller-Suur. As indicated in Fig. 11, two leak sites of potassium play an important role in potassium recycling, i.e., the passive exit of potassium from the medullary collecting duct and the reentry of potassium ions into the descending limb of Henle's loop. They will not be considered further in our discussion (see Chapter 7 by Jamison and Müller-Suur).

A. Potassium Reabsorption across the Proximal Convoluted Tubule

Figure 12 shows a cell model of a proximal tubule cell with those components of potassium transport that account for the translocation of potassium ions from

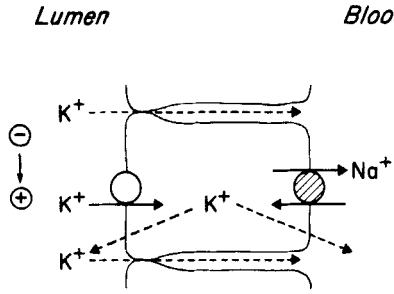

FIG. 12. Cell model of potassium transport across the proximal tubule. Broken arrows indicate passive movement, solid lines indicate active transport. Note change of transepithelial potential from lumen-negative to lumen-positive values, corresponding to the early and middle parts of the proximal convoluted tubule. [From Field *et al.* (1985) and Wright and Giebisch (1985).]

the tubule lumen to the peritubular fluid (Wright and Giebisch, 1985). We note the active sodium–potassium exchange pump in the basolateral cell membrane and a significant potassium conductance, elements of the pump-leak system having already been discussed. Net transport of potassium ions from lumen to peritubular fluid takes place along two routes: *through* cells via active potassium transport in the apical cell membrane and passive exit across the basolateral cell membrane, and *between* cells along a paracellular pathway. The driving force for the latter mode of transepithelial potassium movement is controlled by the magnitude and direction of the transepithelial electrochemical potential difference for potassium. The electrical potential difference is lumen negative in the early tubule, but becomes lumen positive in the middle and late segments of the proximal convoluted tubule. Passive potassium reabsorption could be driven by the lumen-positive potential and by the modest increase in potassium concentration that has been observed along later segments of the proximal tubule.

The notion that a component of active potassium transport is also present in proximal tubule cells is based on three lines of evidence. First, the fact that the early proximal convoluted tubule maintains a lumen-negative potential and a potassium concentration not significantly exceeding that in plasma requires an active driving force to energize potassium reabsorption. Second, the dissociation between net sodium and potassium reabsorption, particularly under conditions in which the lumen-positive potential difference is sharply attenuated (acetazolamide administration), makes it likely that an active transport component must have been involved in potassium reabsorption (Beck *et al.*, 1973).

Finally, a number of experiments suggest that active potassium transport may be present in the apical cell membrane of proximal tubule cells. First, the intracellular potassium activity is above electrochemical equilibrium (Lang *et al.*, 1983; Teulon and Anagnostopoulos, 1982a; Sackin and Boulpaep, 1981; Kubota

et al., 1983a,b; Matsumura *et al.*, 1984b; Edelman *et al.*, 1978; Cemerikic *et al.*, 1982; Biagi *et al.*, 1981a; Sackin and Boulpaep, 1983). Given the presence of a finite, albeit small, potassium permeability in the apical cell membrane (Lapointe *et al.*, 1984; Frömter, 1979; Kawahara, 1985; Kawahara *et al.*, 1985a), potassium ions will continuously leak from cell to lumen. Accordingly, transport processes must be operative to offset this tendency of potassium secretion. These may be either active potassium reabsorption, as indicated in Fig. 10, or, alternatively, potassium movement through the intercellular shunt pathway.

In the amphibian proximal tubule, ouabain leads to a significant increase of the luminal potassium concentration; at the same time, the potassium equilibrium potential across the apical cell membrane declines toward the transmembrane voltage across the basolateral cell membrane. These data demonstrate directly the presence of active potassium transport from lumen into the cell (Fujimoto *et al.*, 1977). Similar studies have not yet been performed in the mammalian proximal tubule; however, the latter is also characterized by cell potassium activity levels above electrochemical equilibrium (Cemerikic *et al.*, 1982; Edelman *et al.*, 1978) and by a significant potassium conductance in the brush-border membrane (Kawahara, 1985).

Two lines of evidence suggest that passive potassium reabsorption may also be present in the proximal tubule. The transepithelial potential difference is lumen positive in the late proximal tubule. This represents a favorable driving force for passive potassium movement across the paracellular shunt pathway. However, it is presently not known, but very likely that the specific potassium conductance of the shunt pathway is high enough to allow significant potassium translocation by this passive reabsorptive mechanism. This is an attractive possibility, since radioactive rubidium, a cation with similar functional behavior as potassium, leaves the lumen at a rate much faster than its rate of washout from proximal tubule cells (Barfuss and Schafer, 1984).

The observation that potassium movement is significantly affected by the rate of transepithelial fluid movement is also relevant. First, it can be shown that maneuvers that suppress proximal net sodium and fluid transport also attenuate net potassium reabsorption (Wright and Giebisch, 1985; Giebisch, 1971). Second, potassium movement across the proximal tubule is also affected by net fluid transport. In perfusion experiments in which not only the rate, but the direction of fluid transport was altered by application of appropriate osmotic gradients, the magnitude and direction of net potassium movement is strongly dependent on that of net fluid movement (Bomsztyk and Wright, 1983, 1986). These results suggest possible solvent drag effects, i.e., direct coupling of fluid to potassium movement through identical transport pathways. To the extent that such solvent drag effects occur by fluid–potassium interactions, they depend on sodium-dependent water transport (Giebisch and Aronson, 1986; Burg, 1985). Part of such fluid movement may occur between cells, and this is the reason why

potassium is depicted in Fig. 12 to move between cells. However, given a high cell water permeability, potassium may also follow water via a transcellular route.

The following is an alternative mode of coupling between fluid and potassium movement. Fluid movement could increase the luminal potassium concentration (solute polarization) and thus create a favorable gradient for passive reabsorption of potassium ions by electrodiffusion. This would require a high cellular or paracellular potassium permeability to allow relatively small concentration gradients to drive potassium ions across the proximal tubular epithelium.

Experiments in perfused rabbit tubules by Barfuss and Schafer (1984) also suggest a paracellular route of potassium translocation. This conclusion is based on the rapid loss of tracer rubidium (a marker for potassium) from the lumen at a time when cell rubidium had not yet disappeared. All mechanisms of passive potassium transport are attractive possibilities, but we do not yet have enough information to evaluate their relative importance. All we can conclude is that active and passive mechanisms participate in retrieving the bulk of the filtered potassium load. However, the relative distribution of cellular and paracellular, of active and passive mechanisms of potassium transport is presently unknown. The proximal tubule is not considered a nephron site where potassium transport is subject to regulation. Accordingly, the cell mechanisms of transport have not attracted wide attention, and our knowledge of the transport operations at this tubular site is limited.

B. Potassium Transport across the Diluting Segment (Thick Ascending Limb of Henle and Amphibian Early Distal Tubule)

The problem of potassium transport across the diluting segment has been extensively studied, and this has led to the development of a cell schema that allows insight into the dual role of potassium—its involvement in net potassium reabsorption and its stimulating role in sodium chloride transport. Useful preparations for study have been the isolated thick ascending limb of the loop of Henle (Greger, 1985; Hebert and Andreoli, 1984; Greger and Schlatter, 1983; Greger *et al.*, 1984; Herbert *et al.*, 1984; Schlatter and Greger, 1984; Teulon and Anagnostopoulos, 1982b; Teulon *et al.*, 1985) and the early distal tubule preparation of the amphibian kidney of *Amphiuma* (Oberleithner *et al.*, 1982b,c, 1983a, 1984, 1985a,b; Oberleithner, 1983, 1985). Remarkably similar results have been obtained in these two preparations.

Figure 13 provides a cell model incorporating the key transport elements that have been identified at this nephron site (Wright and Giebisch, 1985; Greger, 1985; Oberleithner *et al.*, 1982a; Hebert *et al.*, 1984). Again, the main driving

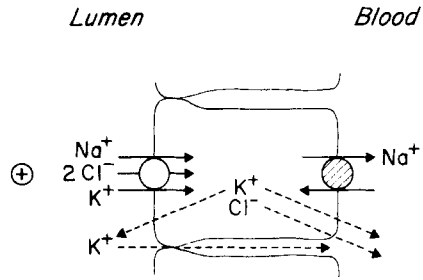

FIG. 13. Mechanisms of potassium transport across the mammalian thick ascending limb and the amphibian early distal tubule. Potassium exit across the basolateral cell membrane may include a component of electroneutral potassium chloride cotransport (not shown). [From Wright and Giebisch (1985).]

force for net potassium reabsorption is ultimately the ATP-dependent, active sodium-potassium exchange in the basolateral cell membrane. This transport operation lowers the cell sodium activity and generates a steep sodium concentration gradient across the apical cell membrane. Energy is thus transferred from the basolateral cell membrane to the apical membrane. It is here that a coupled cotransport mechanism involving one sodium, one potassium, and two chloride ions has been identified. Given its electroneutral character, both sodium and chloride ions diffuse along favorable concentration gradients from lumen into the cell. These chemical gradients are quite adequate to provide energy for a modest uphill movement of potassium, of the order of some 20 mV, across the apical cell membrane. The apical cotransport mechanism is highly sensitive to loop diuretics such as furosemide and bumetanide.

The information in support of the cellmodel of the diluting segment (see Figs. 13 and 14) is based on several lines of evidence (Wright and Giebisch, 1985; Greger, 1985; Oberleithner *et al.*, 1982b,c, 1983a; Greger *et al.*, 1984; Hebert *et al.*, 1984). Briefly, net reabsorption of sodium, potassium, and chloride is sensitive to the deletion from the lumen of any of the three cotransported ions. Loop diuretics, particularly furosemide, have similar effects. Both interventions also abolish the lumen-positive potential difference. Cell ion activities of sodium and chloride are also affected when the apical cotransporter is blocked. Figure 15 summarizes relevant results from experiments in the amphibian diluting segment (Oberleithner *et al.*, 1982b). It is apparent that both cell sodium and chloride activities fall sharply when their entry into the cell is compromised—either by furosemide or by deletion of those ion species that are transferred by the cotransport operation. This effect is consistent with inhibition of entry of sodium and chloride across the apical cell membrane, but inconsistent with continued extrusion of these ions across the basolateral cell membrane.

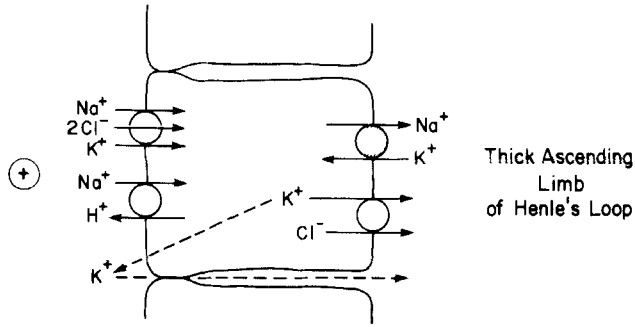

FIG. 14. Expanded cell model of the mammalian (rat) thick ascending limb and the amphibian early distal tubule. Note the presence of an electrically neutral sodium–hydrogen exchanger in the apical cell membrane. Sodium–hydrogen exchange is inhibited by amiloride and, in the amphibian kidney, stimulated by states of hyperaldosteronism. [Based on data by Oberleithner *et al.* (1982a, 1984) and Good *et al.* (1985).]

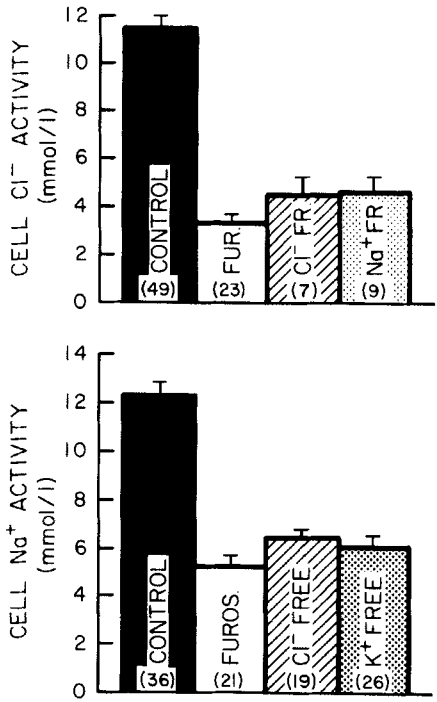

FIG. 15. Ion activities of early distal tubule cells of *Amphiuma*. Furosemide was used at concentrations of 5.10–5 mol/liter, gluconate was used to replace chloride, and bis(2-hydroxyethyl)dimethylammonium was used to substitute for sodium ions in the luminal perfusion fluid. [From Oberleithner *et al.* (1982a).]

Consideration of the passive conduction pathways is also of interest. In the apical cell membrane, potassium ions are the main ion species that cross this membrane by electrodiffusion. Preliminary results using patch-clamp techniques have also identified single channels that are potassium selective (Kawahara *et al.*, 1985b). The apical potassium conductance is reduced after exposure to barium (Davis and Finn, 1982; Oberleithner *et al.*, 1983a; Hebert *et al.*, 1984), after acidifying the bath and lumen medium (Stanton *et al.*, 1982), and following luminal application of amiloride (Oberleithner *et al.*, 1985a).

The effect of amiloride on potassium movement demonstrates an interesting linkage between potassium transport and hydrogen ion secretion at this nephron site. Studies in the isolated mammalian thick ascending limb of the rat have identified an amiloride-sensitive sodium–hydrogen exchange mechanism at this site (Good *et al.*, 1985). A similar process has also been identified in the early distal tubule of *Amphiuma* and been shown to be stimulated by aldosterone (Oberleithner *et al.*, 1984; Oberleithner, 1985). Figure 14 illustrates the presence of this sodium–hydrogen exchanger in an expanded version of the cell model of the diluting segment.

Two functional consequences of the presence, in parallel, of these cotransport (Na^+,2 Cl^-,K^+) and countertransport (Na^+–H^+) mechanisms *and* of a significant potassium permeability in the apical cell membrane are of interest. First, inhibition of the sodium–hydrogen exchanger by amiloride mimics the effects of acidifying the peritubular fluid in that it also reduces the apical potassium permeability (Oberleithner *et al.*, 1984). It is virtually certain that this is due to blocking hydrogen extrusion and to cell acidification. Thus, both direct acidification of the extracellular medium, and amiloride, reduce the apical potassium permeability (Stanton *et al.*, 1982; Oberleithner *et al.*, 1984).

A second phenomenon concerns the effects of inhibition of the Na^+, 2 Cl^-,K^+ cotransporter upon tubular acidification. The relevant observation here is that those diuretics that block the cotransporter acidify the tubular fluid (Hropot *et al.*, 1985; Oberleithner, 1985). In a subsequent discussion, it will be shown that the apical cotransport mechanism is the main pathway for sodium entry into tubule cells of the diluting segment and that its inhibition sharply lowers cell sodium activity (Oberleithner *et al.*, 1983a). Given the presence of the sodium–hydrogen ion exchanger in the same cell membrane as that of the Na^+,2 Cl^-,K^+ cotransporter, it is clear that sodium–hydrogen ion exchange will be stimulated by the steeper sodium concentration gradient that develops across the apical cell membrane as sodium entry by cotransport is blocked.

The potassium conductance in the apical cell membrane is also responsible for the appearance of net potassium secretion that has been observed after blocking of the apical Na^+,2 Cl^-,K^+ cotransporter (Oberleithner *et al.*, 1982b). The two control elements of apical potassium transfer—secondary active potassium movement from lumen to cell, and passive egress of potassium from cell to

lumen—are normally poised toward net potassium reabsorption. However, administration of loop diuretics or replacement of luminal chloride by nonchloride anions leads to effective blocking of the cotransporter and to cessation of potassium reabsorption. Such interventions do not affect the apical potassium conductance. As a consequence, potassium ions now leak unopposed from cell to lumen, and potassium secretion may now become apparent.

The potassium conductance in the apical cell membrane is subject to control by acid–base balance, by the state of potassium balance (at least in the amphibian nephron), and by vasopressin. We have already mentioned that, as shown in studies on isolated, perfused early distal tubules of *Amphiuma* and of the frog, acidification of the peritubular environment reduces the potassium conductance of the luminal cell membrane (Stanton *et al.*, 1982; Oberleithner *et al.*, 1985a). This effect could play a role in regulating potassium excretion in the amphibian nephron, since this nephron segment is characterized by a high intrinsic potassium permeability of the luminal cell membrane, a prerequisite for potassium secretion.

The potassium conductance of the luminal cell membrane has also been evaluated in the amphibian diluting segment in the state of potassium adaptation, i.e., during exposure to high ambient potassium concentrations (Oberleithner *et al.*, 1982b). When the potassium concentration in the water is raised, potassium reabsorption ceases in the early distal tubule of *Amphiuma* and potassium secretion appears (Oberleithner *et al.*, 1982b). Analysis of the fractional and absolute electrical resistance of the apical cell membrane shows a significant fall of the membrane resistance, and this can be shown to be due to a doubling of the absolute potassium conductance (Oberleithner *et al.*, 1985b). Thus, the increase of net potassium secretion coincides with significant augmentation of the apical membrane conductance. Quantitative estimates in these potassium-adapted tubules suggest that this increase in apical potassium conductance suffices to account for the observed rates of net potassium secretion.

Vasopressin can also increase the apical potassium conductance in the diluting segment, as demonstrated by Hebert and his associates in the mammalian thick ascending limb of Henle (Hebert *et al.*, 1984). Coincident with the increase in basolateral chloride conductance (Greger, 1985; Schlatter and Greger, 1984), these events result in stimulation of net sodium chloride transport in the medullary thick ascending limb of Henle (Greger, 1985; Hebert and Andreoli, 1984; Hebert *et al.*, 1984; Schlatter and Greger, 1984). Vasopressin also enhances net potassium secretion in the perfused distal tubule of the rat *in vivo* (Field *et al.*, 1984a) and in the perfused cortical collecting tubule of the rat (Tomita *et al.*, 1985; Reif and Schafer, 1986). In the latter preparation, vasopressin-induced potassium secretion could be blocked by luminal barium. It is likely that stimulation of potassium secretion by antidiuretic hormone is due to the opening of potassium channels in the luminal cell membrane. Patch-clamp experiments in a

culture preparation of chicken kidney cells show activation of potassium channels by vasopressin and thus support the interpretation (Guggino *et al.*, 1985b).

Potassium ions also play a key role in sustaining sodium chloride cotransport in the thick ascending limb of the mammalian loop of Henle and the amphibian diluting segment. Thus, recycling of potassium ions across the apical cell membrane provides a continuous supply of potassium ions for the luminal cotransport mechanism. Quantitative estimates of the extent to which potassium ions recycle across the apical cell membrane are shown in Fig. 16 (Hebert and Andreoli, 1984). This process is important because it allows continuous turnover of the apical cotransporter despite the fact that the sodium and chloride concentrations in the lumen are at least 20 times higher than the corresponding potassium concentration. Clearly, if extensive recycling of potassium ions were not to occur, the luminal supply of potassium would be quickly exhausted and lead to cessation of sodium chloride cotransport. It is the high potassium conductance of the apical cell membrane that safeguards the adequate supply of potassium ions by allowing recycling of potassium ions across the luminal membrane.

The membrane permeabilities to potassium in the apical and basolateral cell membranes of the diluting segment also contribute importantly to the generation of the cell potential and the transepithelial potential difference. Different ionic permeabilities reside within the apical and basolateral cell membrane: Whereas

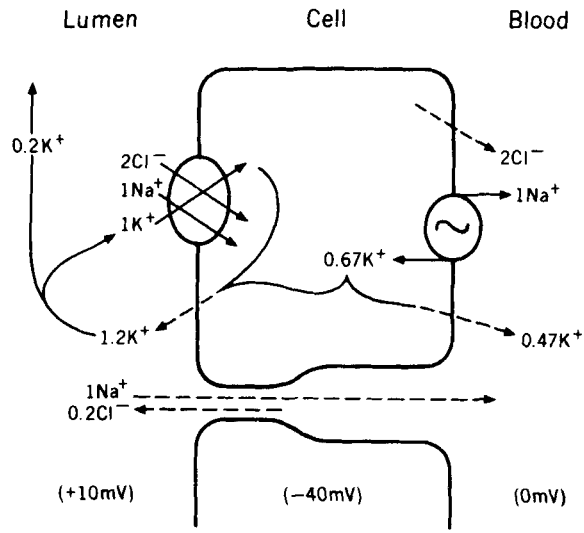

FIG. 16. Cell schema of mammalian thick ascending limb summarizing individual flux components of transported ion species. Note extensive recycling of potassium ions across the apical cell membrane. [From Hebert and Andreoli (1984).]

the apical cell membrane is potassium selective, that of the basolateral membrane is permeable both to potassium and chloride ions (Greger, 1985; Greger and Schlatter, 1983; W. Guggino, 1986; Oberleithner *et al.*, 1982b). It is also well established that the potassium concentration gradients between cytoplasm and extracellular fluid exceed that of chloride (Oberleithner *et al.*, 1982d; Greger, 1985). Because the voltage across the basolateral membrane is the sum of the equilibrium potentials of chloride *and* potassium, the basolateral potential difference is lower than that across apical cell membrane. In the latter, the potential is exclusively determined by the potassium equilibrium potential. Potassium ions thus contribute to setting the cell negative potential. The latter facilitates passive chloride extrusion by electrodiffusion across the basolateral cell membrane.

An important consequence of the unequal polarization of the luminal and basolateral membranes is the lumen-positive transepithelial potential (Greger, 1985; Hebert and Andreoli, 1984; Oberleithner *et al.*, 1982b; Oberleithner, 1983; Planelles and Anagnostopoulos, 1982). It plays an important role in mediating the passive reabsorption of sodium and potassium ions via the intercellular shunt pathway, and it is also largely responsible for the passive reabsorption of bivalent cations (Greger, 1985).

Some evidence also suggests participation of a nonconductive potassium transport pathway in the basolateral cell membrane of the rabbit thick ascending limb (Greger, 1985; Greger and Schlatter, 1983) and the amphibian diluting segment (Guggino, 1986). In the mammalian tubule preparation, the key evidence is interference by barium ions with potassium movement *without* the expected resistance changes in the basolateral cell membrane (Greger and Schlatter, 1983). In the amphibian diluting segment, measurements of both cell volume and cell potentials, after selective ionic substitutions in the basolateral fluid, have shown functional heterogeneity (Guggino, 1986). In one cell type, concentration changes of potassium and chloride in the bath led to membrane voltage alterations expected from the presence of large potassium and chloride conductances. In sharp contrast, another group of cells was much less sensitive to these ion changes, and the membrane voltage changes were relatively small. The resistance ratio, an index of the relative electrical resistance of the apical and basolateral cell membrane, also showed significant differences in the two cell types. Whereas it was high in the cells having high electrical conductance in the basolateral cell membrane, it was sharply lower in the second cell type where the difference between the ionic conductances of the apical and basolateral cell membranes is much smaller.

Results of experiments of cell swelling also support the presence of electroneutral potassium chloride cotransport in one group of cells in the early distal tubule of *Amphiuma* (Guggino, 1986). Significant cell swelling could be induced after raising the potassium concentration in the peritubular fluid. This is significant because of the absence of any evidence for an adequately large elec-

trodiffusive pathway in the basolateral membrane of this cell preparation. Furthermore, cell swelling was significantly reduced after deletion of peritubular chloride. Such behavior would be expected from a chloride-dependent electroneutral pathway for potassium translocation. It is also interesting that the number of cells in which potassium movement was thus shown to be largely electroneutral increased significantly when animals were exposed to a high potassium environment. The precise functional role of this adaptive process needs further study.

C. Potassium Transport across the Late Distal Tubule (Initial Collecting Tubule) and across the Cortical Collecting Tubule

A large body of experimental evidence, based on free-flow micropuncture studies *in vivo* in the rat, has firmly established the late distal tubule (initial collecting tubule) as one of the main sites of potassium secretion (Wright and Giebisch, 1985; Giebisch, 1971; Stanton and Giebisch, 1982; Malnic *et al.*, 1964, 1966a,b, 1971; Wright *et al.*, 1971). So far, it has not been possible to carry out detailed electrophysiological studies on this nephron segment. On the other hand, studies on isolated single mammalian collecting tubules *in vitro* have allowed measurements of net potassium fluxes and transmembrane voltages in single cells (Koeppen *et al.*, 1983; Koeppen and Giebisch, 1984, 1985; O'Neil and Sansom, 1984a,b; Sansom and O'Neil, 1985). Since the early work of Grantham *et al.* (1970), the cortical collecting duct has been identified as an important site of potassium secretion.

It is well established that two distinctive cell types line both nephron segments that secrete potassium. Principal *and* intercalated cells are found in the late distal tubule (initial collecting tubule) and the cortical collecting tubule, and there is strong evidence that the principal cell population is involved in potassium secetion in both nephron segments (Stanton and Giebisch, 1982; Stanton *et al.*, 1981; Stanton, 1985; Kaissling, 1982; see also Chapter (11). Furthermore, it is assumed that the intrinsic properties of the principal cells lining the late distal tubule and the cortical collecting duct are similar. We shall now proceed to the discussion of relevant transport operations. Some historical developments are of interest.

The first cell model of potassium secretion was proposed by Berliner and his associates (Berliner, 1961) to explain experimental data obtained in a series of fundamental clearance experiments in which they observed, in particular, the sensitivity of potassium excretion to the sodium content in the urine (Davidson *et al.*, 1958).

Figure 17 incorporates a sodium–potassium exchange pump in the apical cell membrane. Such exchange of sodium for potassium is an important element of

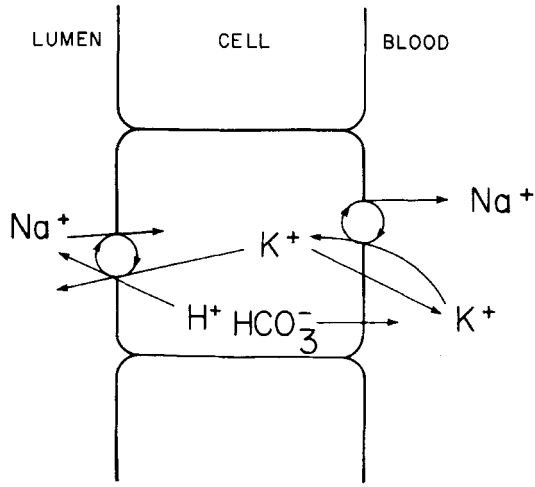

FIG. 17. Cell schema of distal potassium secretion and its dependence on sodium exchange. [From Berliner (1961).]

the original cell model of Berliner (1961). The idea that potassium secretion was coupled to sodium reabsorption was based on three observations: (1) a marked stimulating effect of sodium ions upon potassium secretion, particularly when sodium excretion is initially maintained at low levels (Davidson *et al.*, 1958); (2) the observation that adrenal steroids enhance both the secretion of potassium as well as the reabsorption of sodium ions, and (3) that no evidence was obtained, consistent with tubular anion secretion, that could account for the amounts of potassium secreted. This latter observation was taken as the strongest evidence of exchange of luminal sodium for cellular potassium (Berliner, 1961). It is for these reasons that a sodium–potassium exchanger was placed in the luminal cell membrane. In addition, the sensitivity of potassium secretion to acid–base disturbances led to the assumption of competition between potassium and hydrogen ions (Berliner, 1961). At the time of inception of this model, no information was available on the electrical profile across the secretory tubule epithelium. When such information was obtained in the 1960s by the application of microelectrode techniques to the study of electrical phenomena in the kidney, it was apparent that the sodium–potassium exchanger had to be localized in the basolateral cell membrane. The respective electrochemical gradients of sodium and potassium ions across the apical cell membrane were of adequate magnitude to allow sodium entry and potassium exit to proceed by diffusion alone.

Figure 18 shows a more recent "distal" cell model where the possible mechanisms for potassium (and sodium) transport are shown (Giebisch *et al.*, 1967).

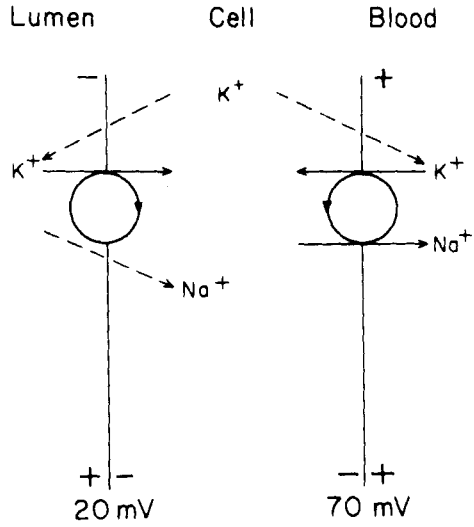

FIG. 18. Cell schema of distal tubule cell with mechanisms for potassium secretion *and* potassium reabsorption. Note presence of a sodium and potassium conductance as well as an active potassium pump in the apical cell membrane. The two cell membranes maintain different electrical potentials such that the apical membrane is depolarized with respect to the basolateral membrane. [From Giebisch *et al.* (1967).]

This model takes into account data on net transport, the electrochemical potential gradients of sodium and potassium across the apical and basolateral cell membranes, permeability properties, and the direction of the transepithelial potential difference.

Key features of this model of potassium transport, first published in 1967 (Giebisch *et al.*, 1967), are summarized in Fig. 18. They include (1) an active, ATP-dependent sodium–potassium exchange in the basolateral cell membrane, (2) a potassium conductance in the basolateral cell membrane, (3) a potassium *and* sodium conductance in the apical cell membrane, and (4) an active reabsorptive potassium pump in the apical cell membrane.

Potassium secretion was envisaged to occur in two distinct steps. First, active potassium uptake across the basolateral cell membrane accumulates potassium ions in the cell. In a second step, potassium ions diffuse from cell to lumen along a favorable electrochemical potential gradient. This latter transport pathway is thought to be entirely passive in nature. Sodium ions also play a significant role in the process of potassium secretion. They diffuse from lumen to cell and depolarize the apical cell membrane. It was thought that this unequal polarization of the two membranes (partial depolarization of the luminal cell membrane) *and*

the high potassium permeability of the luminal membrane were essential transport components of potassium-secreting cells.

With evidence accumulating that two cell types are present in the late distal tubule (initial collecting tubule) and cortical collecting tubule, it became likely that these two cell populations could subserve different transport functions. This possibility became even more attractive with the emergence of morphological data demonstrating significant fine structural changes in the apical cell membrane of intercalated cells in conditions of experimental hypokalemia (Toback *et al.*, 1976; Stetson *et al.*, 1980). The postulate for active potassium reabsorption is based on micropuncture experiments on the distal tubule in the rat (Malnic *et al.*, 1966a; Duarte *et al.*, 1971). This condition is characterized by complete absence of net potassium secretion, yet persistence of a distinctly lumen-negative transepithelial potential difference. In the absence of other transport mechanisms, potassium ions should be secreted into the lumen by the electrical potential. To prevent such potassium secretion, an active force must move potassium ions out of the lumen; hence, the suggestion of an active mechanism of potassium reabsorption and its localization in the apical cell membrane (Giebisch, 1971; Malnic *et al.*, 1966b).

Little is known about the characteristics of active potassium transport from lumen into the cytoplasm of intercalated cells. No information is available as to whether this transport is electrogenic, whether it proceeds by cotranport with other ions, or whether it represents exchange with other cations. Since intercalated cells also show marked fine structural changes following acid–base disturbances in which hydrogen ion secretion is stimulated (Madsen and Tisher, 1983), the possibility should be considered that potassium reabsorption and hydrogen ion secretion are coupled by a carrier-mediated exchange mechanism. It is not known whether this putative exchanger is an active, ATP-dependent process. If not, this raises the problem of an adequate energy source because potassium reabsorption across the luminal cell membrane has to proceed against a steep concentration gradient. The magnitude and direction of the hydrogen ion gradient would not be sufficient for potassium movement to take place by electroneutral exchange for hydrogen ions. It is of interest that active potassium reabsorption has been observed in the colon (see Chapter 21) and in the turtle bladder (Husted and Steinmetz, 1981, 1982).

Possibilities of the differential distribution of transport parameters that could account for different modes of potassium transport are summarized in Fig. 19, which depicts the hypothetical distribution of relevant transport components in a reabsorbing cell (intercalated cell) and a secretory cell (principal cell) (Stanton and Giebisch, 1982). Main differences that could account for potassium reabsorption and potassium secretion in the two cell types are thought to be twofold: absence of a significant potassium permeability, and presence of the reabsorptive

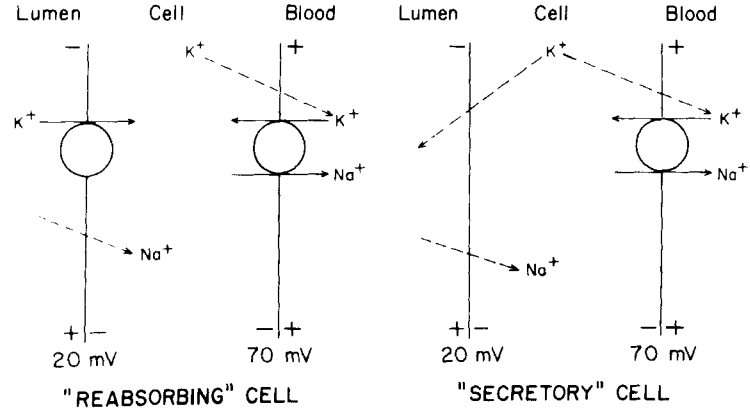

FIG. 19. Two-cell system for potassium reabsorption and potassium secretion. Note modifications of the transport mechanisms of the apical cell membrane in the two cell types subserving control of potassium handling along the nephron. Strong evidence suggests that the principal cell secretes potassium ions and that the intercalated cell reabsorbs potassium. [From Stanton and Giebisch (1982).]

potassium pump in the potassium-reabsorbing cell. In contrast, the presence of a high potassium permeability and absence of the active reabsorptive potassium pump could explain the functional behavior of the secretory cell.

An extensive series of electrophysiological studies has been carried out over the past few years in the isolated, perfused rabbit cortical collecting tubule *in vitro*. They have significantly contributed to our understanding of the mechanisms controlling potassium secretion. In particular, the work of Koeppen *et al.* (1983; Koeppen and Giebisch, 1984, 1985) and of O'Neill and Samson (1984a,b, 1985) has added significantly to our knowledge of the transport properties of the apical and basolateral cell membrane of single collecting tubule cells. Methods have also been devised to distinguish between principal and intercalated cells by electrophysiological and optical criteria (O'Neil and Hayhurst, 1985). In addition, *in vivo* perfusion studies of the superficial distal tubule in the rat have also contributed to a better understanding of the cell mechanism that controls potassium transport (Good and Wright, 1979; O'Neil and Boulpaep, 1979).

These newer studies have all confirmed the basic features of the original model of potassium secretion which was proposed to explain potassium secretion (and reabsorption) in the distal tubule (initial collecting tubule) (Giebisch *et al.*, 1967). Figure 20 summarizes the relevant transport mechanisms suggested for principal cells.

1. In the basolateral membrane, active potassium uptake and active sodium extrusion, driven by ATP, is the main transport element. The active uptake step

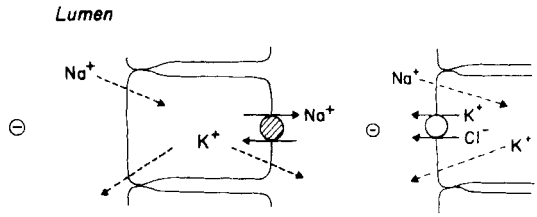

FIG. 20. Potassium transport in cells of the late distal tubule (initial collecting tubule) and cortical collecting tubule. The left panel shows potassium exit across the basolateral cell membrane by electrodiffusion, the right panel by cotransport of potassium and chloride ions. [From Wright and Giebisch (1985).]

responds to changes in acid–base disturbances, changes in plasma potassium levels, and variations in circulating levels of mineralocorticoid hormones (Wright and Giebisch, 1985; Giebisch, 1971). From single cell impalements with conventional microelectrodes, it can be deduced that the sodium–potassium pump operates in an electrogenic mode, particularly after increasing the level of circulating mineralocorticoid steroids. Such stimulation of electrogenic sodium–potassium exchange plays an essential role in the very dramatic electrical hyperpolarization of these tubule cells which follows pump stimulation by aldosterone and desoxycorticosterone acetate (Koeppen *et al.*, 1983; Koeppen and Giebisch, 1984, 1985; Sansom and O'Neil, 1985).

2. A potassium conductive pathway is also present in the basolateral cell membrane. This property, shown in Fig. 20, generates a diffusion potential for potassium ions from cell to peritubular fluid. This potassium pathway in the basolateral cell membrane has also been shown to play a role in the secretory response of potassium ions to mineralocorticoid stimulation (Koeppen and Giebisch, 1984, 1985; Sansom and O'Neil, 1985). Typically, the basolateral potassium conductance increases after mineralocorticoid treatment at a time when the basolateral cell membrane also responds with a sharp hyperpolarization. As the electrogenic stimulation of the sodium–potassium pump drives the membrane potential above the potassium equilibrium potential, potassium ions are then driven passively from the peritubular fluid into the cell. This contrasts with the situation under control conditions when the membrane potential is below the potassium equilibrium potential and potassium ions leak from cell to peritubular fluid. This passive potassium uptake across the basolateral cell membrane after mineralocorticoid treatment thus constitutes an additional pathway for cell potassium uptake, in parallel with active, pump-mediated potassium uptake. The overall effect of this reversal of passive movement of potassium is to make the transepithelial potassium secretion more efficient.

3. The apical cell membrane also has a significant potassium permeability. This highly potassium-selective pathway provides an important route for po-

tassium secretion from cell to lumen (see Fig. 21). Passive in nature, this transport step has been shown to respond to changes in the electrochemical potential gradient of potassium ions. Accordingly, lowering the potassium concentration in the lumen stimulates potassium secretion (Wright and Giebisch, 1985; Good and Wright, 1979) by steepening the potassium concentration difference between cytoplasm and luminal fluid. Changes in luminal membrane potential, brought about by external current application, also affect potassium secretion in the expected manner: Increasing the lumen-negative potential stimulates potassium secretion (Garcia-Filho *et al.*, 1980). Amiloride, known to block potassium secretion in the distal tubule (Duarte *et al.*, 1971) as well as in the cortical collecting tubule (Stoner *et al.*, 1974), acts by hyperpolarizing the apical cell membrane and thus impedes passive egress of potassium from cell to lumen (O'Neil and Boulpaep, 1979). This effect is mediated by the blocking action of amiloride on the apical sodium conductance of potassium-secreting cells.

The potassium permeability of the luminal cell membrane plays a significant role in regulating potassium secretion.The potassium permeability of the luminal membrane is increased after mineralocorticoid administration (Koeppen and Giebisch, 1984, 1985; Sansom and O'Neil, 1985) and reduced after luminal acidification (O'Neil, 1983). It is also sensitive to the inhibitory action of luminal barium (Koeppen *et al.*, 1983; O'Neil and Sansom, 1984a). By directly measuring the potassium permeability of the apical cell membrane of isolated, perfused rabbit collecting tubules, Muto *et al.* (1986) have also shown that changes in potassium intake, *independent* of circulating levels of aldosterone, have significant effects. Exposure to a low potassium diet blocks the luminal potassium conductance, whereas administration of a high potassium diet has the opposite effect. It is most likely that diet-induced alterations in the cell potassium

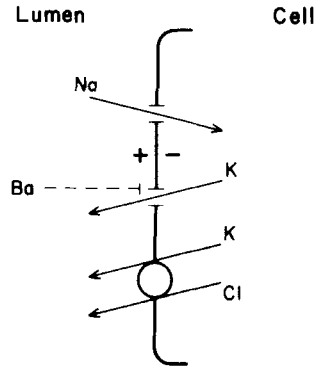

FIG. 21. Schema of apical cell membrane of potassium secretory cell. Two modes of potassium transfer are shown: (1) potassium translocation by diffusion (barium-inhibitable), and (2) by electroneutral potassium chloride cotransport. [Based on data by Ellison *et al.* (1985).]

activity per se affect the permeability of the apical cell membrane to potassium ions.

Experiments by Hunter *et al.* (1984), by Koeppen and Hellman (1984), and by Palmer and Frindt (1987) have recently demonstrated the presence of single potassium channels in the apical cell membrane of isolated, cortical rabbit collecting tubules. The main characteristics of this potassium channel are summarized in Table III. Of particular interest is the marked voltage barium and calcium dependence of channel activity.

Figure 22 shows single-channel activity in an excised patch of the apical cell membrane and the stimulating effect of calcium ions (Hunter *et al.*, 1984). The voltage sensitivity also deserves attention. As the luminal membrane depolarizes, open potassium channel activity increases. This effect is of interest because it may explain the observed enhancement of the apical potassium conductance that follows mineralocorticoid stimulation. Administration of this mineralocorticoid lowers the apical membrane potential (Koeppen *et al.*, 1983; Koeppen and Giebisch, 1984, 1985; Sansom and O'Neil, 1985), and it is attractive to speculate that the voltage dependence of the observed single potassium channel mediates the increase in potassium permeability of the luminal cell membrane.

4. The apical cell membrane is also the site of a significant sodium permeability. The sodium permeability plays an important role in potassium secretion. First, a sodium gradient from lumen to cell favors diffusion of sodium ions. This generates a diffusion potential that opposes the potassium diffusion potential from cell to lumen. Compared to the basolateral cell membrane that has an inherently low sodium conductance (and where sodium ions are thus relatively ineffective in reducing the potassium diffusion potential), the apical membrane potential is smaller than the voltage across the peritubular cell membrane. Since the apical membrane depolarization favors passive potassium secretion across the apical cell membrane, sodium ions are an important element in the process of potassium movement from cell to lumen. Second, the sodium permeability mediates entry of this ion by electrodiffusion. Unsaturated over a wide range of distal tubule flow rates (Khuri *et al.*, 1975b), it allows stimulation of net sodium

TABLE III

K^+ CHANNEL CHARACTERISTICS FROM APICAL MEMBRANE OF CORTICAL COLLECTING TUBULE

Conductance = 40 pS
Selectivity K^+:NA^+ = 9:1
Ca^{2+}-activated
Voltage dependent: Depolarizing potentials increase the open probability
Inhibited by barium: Apparent K_i = 12 μMM

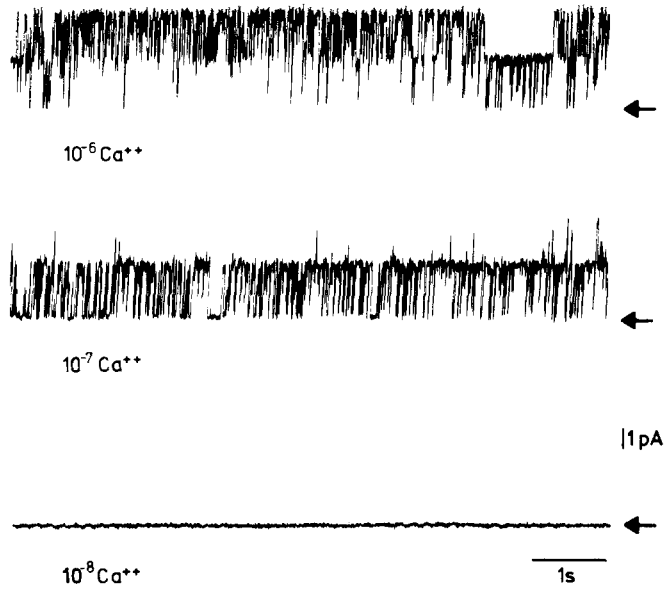

FIG. 22. Calcium activation of potassium channel in the apical cell membrane of cortical collecting tubule cell. Inside-out patch configuration. [From Hunter *et al.* (1984).]

reabsorption with increased delivery of fluid and sodium ions into the late distal tubule and into the cortical collecting tubule. In free-flow micropuncture studies of distal tubules, this enhancement of sodium transport is mediated by the increases of the sodium concentration along the distal tubule as flow rate rises (Khuri *et al.*, 1975b). Since potassium secretion also increases with augmentation of flow rate (Khuri *et al.*, 1975a), sodium entry into principal cells would provide the appropriate stimulus for increased activity of the basolateral sodium–potassium exchange pump.

5. Evidence based on microperfusion studies in superficial rat distal tubules is consistent with an additional secretory pathway for potassium across the apical cell membrane. The right panel of Fig. 20 defines a neutral cotransport mechanism of potassium and chloride ions in the apical cell membrane of principal tubule cells (Ellison *et al.*, 1985). The evidence in support of this mechanism is the stimulation of net potassium and unidirectional ^{42}K tracer fluxes into the lumen when the tubule is perfused with low chloride solutions. This component of potassium secretion is not associated with alterations in the transepithelial potential difference and, importantly, is not blocked by barium. It is not clear whether this mechanism of potassium transport nromally contributes to potassium secretion or whether it is dormant and activated only when the chloride concentration in the lumen falls to low levels (Ellison *et al.*, 1985).

V. EFFECTS OF HORMONES ON CELL MECHANISMS MEDIATING POTASSIUM SECRETION

Information is available on the cellular response of potassium secretory cells to two hormones, mineralocorticoids and vasopressin. Both stimulate potassium secretion.

The effects of aldosterone and of DOCA on the apical and basolateral components of potassium transport have been examined in considerable detail in principal cells of the isolated cortical collecting tubule. This topic will be discussed by O'Neill in Chapter 9. Essentially, the stimulation of potassium transport by mineralocorticoids involves direct effects on *both* the basolateral and the apical components of the sodium and potassium transport systems (Koeppen and Giebisch, 1984; Sansom and O'Neil, 1985). Table IV provides relevant information. The effects upon fine structure and the enzymatic changes (ATPase) that occur in the distal nephron during chronic exposure to mineralocorticoids will be discussed in Chapter 11 by Stanton.

Mineralocorticoids are not the sole class of adrenal hormones that stimulate potassium secretion. The rise in potassium excretion following glucocorticoid administration is solely mediated by augmentation of flow rate along the tubular site of potassium secretion. When distal tubules are perfused *in vivo* at constant flow rates, glucocorticoids have no stimulatory effect on potassium secretion (Field *et al.*, 1984b). Nevertheless, the hormone can be shown to be distinctly kaliuretic, but this effect is associated with enhancement of urine flow rate. Induction of diuresis is a typical effect of glucocorticoids and itself an effective stimulus of potassium secretion (Stanton *et al.*, 1984).

Antidiuretic hormone also stimulates potassium secretion. This effect can be demonstrated in perfused distal rat tubules *in vivo* (Field *et al.*, 1984a) and in perfused cortical collecting tubules of the rat (Tomita *et al.*, 1985; Reif and Schafer, 1986; Schafer and Troutman, 1986).

The most likely mechanism of action of antidiuretic hormone is schematically summarized in Fig. 23. The available evidence suggests strongly that vas-

TABLE IV

MECHANISM OF ACTION OF MINERALOCORTICOIDS ON RENAL K^+ TRANSPORT[a]

1. Increase in basolateral Na^+–K^+ pump activity
2. Increase in cell negativity
3. Increase in apical Na^+ permeability
4. Increase in lumen-negative potential
5. Increase in apical K^+ permeability
6. Increase in basolateral membrane area and of ATPase in principal cell population

[a] Based on data by Koeppen and Giebisch (1984), and O'Neil and Samson (1985).

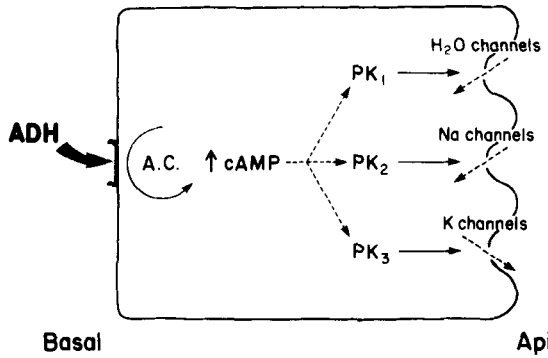

FIG. 23. Cell schema of action of antidiuretic hormone on potassium secretion. Note three distinctive actions of the hormone, on water, sodium, and potassium transport across the apical cell membrane. The possibility is also shown that three cell messengers (cyclic AMP) are involved. [From Field *et al.* (1984a).]

opressin increases the potassium permeability of the apical cell membrane (Field *et al.*, 1984a). This view is based on two lines of evidence. First, in the perfused rat cortical collecting tubule the stimulation of potassium secretion following addition of vasopressin can be blocked by barium, a known inhibitor of luminal potassium conductances (M. C. Reif and J. A. Schafer, personal communication). Second, single-channel recordings from cell-attached patches of apical membranes of isolated chick kidney cells indicate the presence of a potassium channel (Guggino *et al.*, 1985b). Its open time could be sharply increased by addition of antidiuretic hormone. Forskolin, an activator of adenylate cyclase, had similar effects. Further properties of this potassium channel include voltage, calcium, and barium sensitivity. These results from patch-clamp studies strongly suggest that activation of a potassium channel by cyclic AMP mediates vasopressin-induced stimulation of potassium secretion in the distal tubule and cortical collecting tubule.

VI. CONCLUSION

Potassium transport in the renal tubule is mediated by the same transport elements identified in other cells of the body. They include primary active uptake of potassium by the sodium–potassium pump, secondary active transport by cotransporters coupling the movement of Na, K, 2 Cl, and K, Cl, and passive diffusion.

Whether potassium is secreted or reabsorbed across the renal tubule depends critically upon the apical cell membrane. The low potassium permeability of the

brush-border membrane of proximal tubule cells minimizes leakage of potassium ions into the lumen. On the other hand, it is the high potassium permeability of a subset of specialized cells—the principal cell—in the distal nephron that conveys upon this nephron structure the ability of potassium secretion.

Regulation of potassium transport is mediated by activation of specific renal and extrarenal stimuli acting on both basolateral and apical potassium transport mechanisms. Effective interaction of transport events between the basolateral and apical cell membrane safeguards the constancy of cell potassium content and cell volume of tubule cells despite large fluctuations of net transport of potassium and sodium ions.

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Chapter 9

Adrenal Steroid Regulation of Potassium Transport

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I. INTRODUCTION

The mammalian collecting tubule system plays a dominant role in regulating potassium excretion by the nephron. It has been shown that the early segments in particular, including the late distal tubule—now known to be the initial collecting tubule—and the cortical collecting duct (Giebisch and Stanton, 1979; Koeppen *et al.*, 1983a; O'Neil, 1981), avidly secrete potassium and are responsible for most of the potassium excreted in the urine, as discussed in other chapters. Today, the cellular mechanisms of potassium secretion have been in part determined for both of these segments, although the underlying mechanisms of regulation are often poorly understood.

Potassium secretion by these early collecting tubule segments is an active process directly linked to active sodium absorption via the Na:K exchange pump located at the basolateral border of the cells. As anticipated, potassium secretion is dependent upon delivery of sodium to, and reabsorption by, the epithelial cells. However, numerous other factors are known to play a role in regulating potassium secretion, including tubular fluid flow rate, acid–base status, alterations in the sodium and potassium balance, and a variety of humoral factors, most notably the adrenal steroids.

The purpose of this chapter is to present work from our laboratory on the cellular mechanisms of action of mineralocorticoids on potassium secretion by the rabbit cortical collecting duct. Since potassium secretion is intimately associated with sodium absorption, the regulation of both ions will be considered.

II. POTASSIUM TRANSPORT PROPERTIES OF THE CORTICAL COLLECTING DUCT

Our understanding of the transport functions of the rabbit cortical collecting duct was greatly enhanced with the development of techniques by Burg and co-workers (Burg *et al.*, 1966) to isolate and perfuse renal tubule segments *in vitro*. Using these techniques, several laboratories have demonstrated that both sodium absorption and potassium secretion by the rabbit cortical collecting duct are active in nature and both are dependent upon the mineralocorticoid status of the animal from which they were derived (Koeppen *et al.*, 1983b; O'Neil and Helman, 1977; Sansom and O'Neil, 1985; Stokes, 1981; Schwartz and Burg, 1978). With elevated plasma mineralocorticoid levels, the tubules have a marked increased capacity to both absorb sodium and secrete potassium, while the converse is true with depressed mineralocorticoid levels. Elucidation of the mechanisms of action of mineralocorticoids in renal cells has not been rapidly forthcoming, in part because of limitations in the methods of studying cellular transport properties of individual tubules and because of the presence of different cell types which may vary in their functions. Indeed, based on morphological and histochemical studies, two main cell types have been identified in the cortical collecting duct—the principal cell, accounting for 70% of all cells, and the intercalated cell, accounting for the remaining 30% (Kaissling and Kriz, 1979; LeFurgey and Tisher, 1979; O'Neil and Hayhurst, 1985a; Wade *et al.*, 1979; Welling *et al.*, 1981).

Recently, some of these limitations have been overcome with the application of microelectrode techniques to the isolated perfused cortical collecting duct both in this laboratory (O'Neil and Sansom, 1984a,b; Sansom and O'Neil, 1985; Sansom *et al.*, 1984) and by Koeppen and co-workers (Koeppen *et al.*, 1983b; Koeppen and Giebisch, 1985). With use of fine-tipped glass microelectrodes back-filled with 0.5 *M* KCl, individual cells could readily be impaled from the bath side (blood side) of the epithelium across the basolateral membrane to directly record the basolateral membrane voltage, V^b. From this and simultaneous measurement of the transepithelial voltage, V^{te}, the apical (luminal) membrane voltage can be calculated (O'Neil and Sansom, 1984b) as

$$V^a = V^{te} - V^b \tag{1}$$

At periodic intervals a current pulse, I_0, was injected across the epithelium via the perfusion pipette. Assuming that the induced current flow across the apical cell border was equal to that across the basolateral border, the resulting voltage deflections, ΔV, across each border would be proportional to the respective barrier resistances so that the fractional resistance of the apical border, fR^a, could be calculated as

$$fR^a = R^a/(R^a + R^b) = \Delta V^a/(\Delta V^a + \Delta V^b) \quad (2)$$

where R^a and R^b are the apical and basolateral membrane resistances, respectively.

A cell was considered to be impaled successfully if the following minimal criteria were met: (1) Upon impalement there was an initial rapid negative voltage deflection which was either maintained or transiently decayed, but then repolarized to a value equal to or more negative than the initial deflection, (2) fR^a was less than one, and (3) upon withdrawal of the microelectrode from the cell, the offset potential was less than 5 mV (O'Neil and Sansom, 1984a,b). Using these criteria, a cell population was observed that had a mean V^b of -70 to -80 mV and fR^a near 0.5. Occasionally, upon attempting to impale a cell, a stable negative V^b was observed near -40 mV, but fR^a was 1. Since these values would be consistent with either a second cell type or a poor impalement in which the cell membrane did not "seal" around the microelectrode tip, the recordings were discarded.

Both Koeppen *et al.* (1983b) and O'Neil and Sansom (1984a,b) have shown that those cells of the cortical collecting duct "successfully" impaled with microelectrodes are characterized by an amiloride-sensitive sodium conductance and barium-sensitive potassium conductance at the apical cell border. At the basolateral cell border, a dominant chloride conductance has been identified that is paralleled with a relatively small barium-sensitive potassium conductance (O'Neil, 1983; O'Neil and Boulpaep, 1982; Sansom *et al.*, 1984). A cellular model depicting some of these properties is given in Fig. 1.

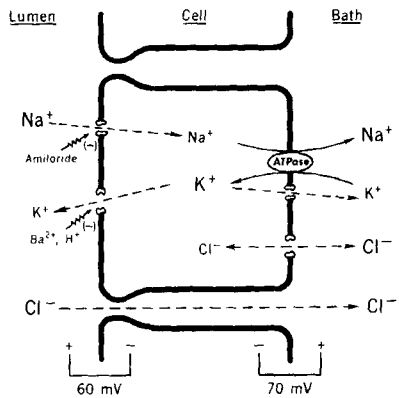

FIG. 1. Cellular model of ion transport for the principal cell of the rabbit cortical collecting duct. The symbol (-) indicates sites of inhibition. See text for details.

III. FUNCTIONAL DIFFERENTIATION OF CELL TYPES USING OPTICAL TECHNIQUES

A. Identification of Cell Types in the Perfused Cortical Collecting Duct

To obtain an understanding of the cellular mechanisms of action by which mineralocorticoids influence sodium and potassium transport in the cortical collecting duct, it is imperative that the role of the two cell types—principal cells and intercalated cells—be delineated. In recent studies in which Hoffman Modulation Contrast optics were employed to view perfused cortical collecting ducts, two cell types were readily apparent based on their gross cell morphology (O'Neil and Hayhurst, 1985a), substantiating the morphological and histochemical data noted above. When viewed face-on, one cell type was characterized by a "hexagonal" profile while the other cell type was characterized by a "circular" profile, as evident in the light micrograph of Fig. 2. To confirm the identity of the cell types, fluorescein-labeled peanut lectin, which has been shown to bind specifically to the luminal surface of intercalated cells (Le Hir *et al.*, 1982b), was added to the perfusate and its binding monitored using epifluorescent techniques. The peanut lectin was observed to bind only to the cells with a circular profile, confirming them as intercalated cells and the cells with a hexagonal profile as principal cells (O'Neil and Hayhurst, 1985a).

B. Ion Transport Properties of the Principal Cell

Once the two cell types could be identified in the perfused tubule, an attempt was made to selectively study their transport properties using microelectrodes techniques. It was found that a microelectrode tip could easily be placed on the basolateral aspect of either cell type; however, only the principal cell could be successfully impaled. The principal cell was observed to have properties of the cell model discussed above (Fig. 1). As shown in Table I, addition of 5 m*M* Ba^{2+} to the luminal perfusate or reduction of the perfusate pH to 4.0 caused a marked depolarization of V^b (and V^a) and increase in fR^a of the principal cell, effects shown previously to arise from blockage of an apical cell membrane K^+ channel (O'Neil, 1983; O'Neil and Sansom, 1984a,b). Alternatively reduction in the bathing medium Cl^- concentration depolarized V^b and decreased fR^a of the principal cell, confirming the presence of a basolateral membrane Cl^- conductance as described previously for the epithelium (O'Neil and Boulpaep, 1979; Sansom *et al.*, 1984). Preliminary evidence was also obtained that the principal cell possessed an amiloride-sensitive Na^+ conductance in the apical cell membrane, a result anticipated from previous studies (O'Neil and Boulpaep, 1979;

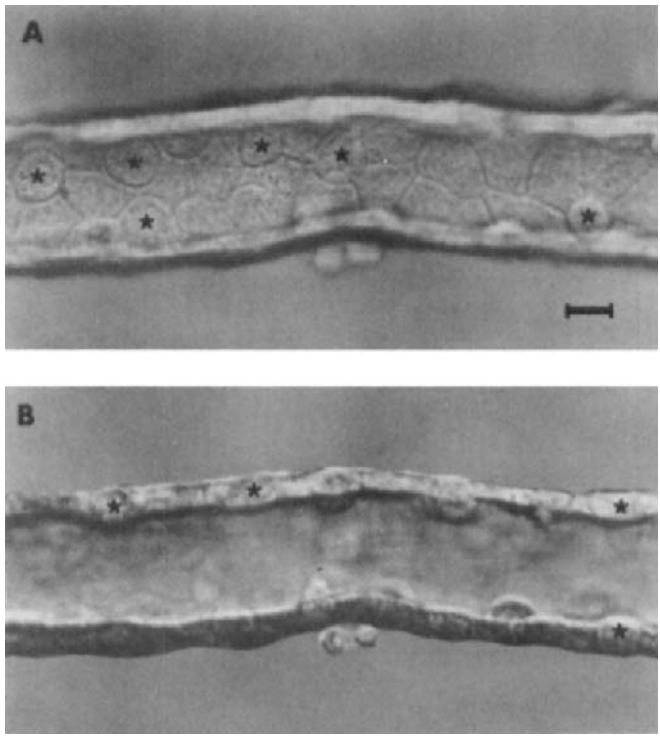

FIG. 2. Hoffman Modulation Contrast images of the perfused cortical collecting duct. (A) Plane of focus on the luminal cell border providing a face-on view of cells. Cells with a hexagonal profile are principal cells and cells with a circular profile (asterisks) are intercalated cells. (B) Plane of focus on the lateral tubule wall providing a cross-sectional view of cells. Cells with flat apical borders are principal cells and cells whose apical borders protrude into the lumen (asterisk) are intercalated cells. See text for methods. Bar = 10 μm. [From O'Neil and Hayhurst (1985a).]

O'Neil and Sansom, 1984a; Sansom and O'Neil, 1985). Further evidence of an apical cell membrane amiloride-blockable sodium conductance was obtained in current-induced cell swelling experiments, as discussed below.

In contrast to the principal cell, the intercalated cell was found to be exceeding difficult to impale. Occasionally a stable impalement was observed, but it was characterized by a low value of V^b near -30 to -40 mV and a high fR^a near 1. Whether this reflects real properties of this cell or impalement damage is not known at present. It is noteworthy, however, that Koeppen (1985) has reported that in the outer medullary collecting duct (inner stripe), cell impalements are characterized by a low V^b and high fR^a, but the basolateral border is chloride permselective, confirming a successful impalement. Since all cells from this

TABLE I
ELECTRICAL PROPERTIES OF THE PRINCIPAL CELL OF THE CORTICAL COLLECTING DUCT

A. Effect of Ba^{2+} addition to perfusate (n = 8)	Control perfusate	+5 mM Ba^{2+}
V^{te}, mV	−8.0 ± 1.7	−8.4 ± 1.6
V^{b}, mV	−78.9 ± 2.3	−37.2 ± 3.0[a]
fR^{a}	0.52 ± 0.11	0.81 ± 0.07[a]
B. Effect of reducing perfusate pH (n = 3)	Control perfusate (pH 7.4)	pH 4.0
V^{te}, mV	−8.8 ± 2.3	−10.4 ± 2.4
V^{b}, mV	−74.3 ± 4.3	−45.0 ± 5.5[a]
fR^{a}	0.52 ± 0.21	0.76 ± 0.18[a]
C. Effect of reducing bathing medium Cl^- concentration (n = 6)	Control bath (154 mM Cl^-)	64 mM Cl^-
V^{te}, mV	−6.8 ± 1.4	−1.7 ± 1.8[a]
V^{b}, mV	−83.3 ± 2.2	−77.5 ± 3.3[a]
fR^{a}	0.54 ± 0.13	0.46 ± 0.14[a]

[a] Significantly different from control values using paired observations, $P < 0.05$.

outer medullary segment are likely acid-secreting cells, it will be interesting to evaluate whether those normally discarded recordings from the cortical collecting duct actually reflect real properties of the acid-secreting cells of this segment—supposedly the intercalated cells.

An alternative approach for examining differences in transport properties of the two cell types of the cortical collecting duct was to utilize the asymmetric permeability properties of the two cell borders of the principal cell determined in the microelectrode studies. As summarized in Fig. 1, the apical cell membrane of the principal cell is cation selective, being permeable to sodium and potassium, while the basolateral cell membrane is anion selective, being permeable primarily to chloride, although a small potassium permeability is present. As a result of this asymmetry, passage of a positive lumen to bath current pulse should cause the cells sharing these properties to swell. The injected positive current would be carried across the apical border by cation movement (sodium and potassium) into the cell, while the positive current would be carried across the basolateral border primarily by anion movement (chloride) into the cell, although a small cell to bath potassium current would be present because of the low potassium permeability at that border. Hence, solute would be added to the cell,

causing swelling. Furthermore, addition of amiloride and barium to the luminal perfusate would block the conductive pathways at that border and thereby abolish the current-induced cell swelling.

This notion was tested previously in isolated tubules perfused and bathed in an NaCl Ringer's solution (O'Neil and Hayhurst, 1985a). Using Hoffman Modulation Contrast optics, a principal cell and intercalated cell were identified in cross section, but adjacent to each other, and the cell height measured as a relative index of cell volume, whereupon a positive lumen to bath current pulse (150 nA) was injected via the perfusion pipette for 10 sec or more. It was observed that current injection caused immediate and marked cell swelling of the principal cells only and that the current-induced swelling could be abolished by simultaneous addition of both amiloride (50 μM) and barium (5 mM) to the luminal perfusate. These studies have recently been extended to examine the separate effects of amiloride and barium, as summarized in Fig. 3. In the absence of luminal amiloride and barium, passage of the positive current pulse for 10 sec caused marked cell swelling of the principal cell only, as previously. The luminal addition of either amiloride (50 μM) or barium (5 mM) reduced cell swelling, but required the simultaneous addition of both amiloride and barium to abolish the current-induced cell swelling of the principal cell. In contrast, the intercalated cell volume was not significantly altered by current passage or addition of amiloride and barium. This provides direct evidence that the transport properties of the two cell types are very different, consistent with our preliminary microelectrode studies and with the notion that the cells are not likely osmotically

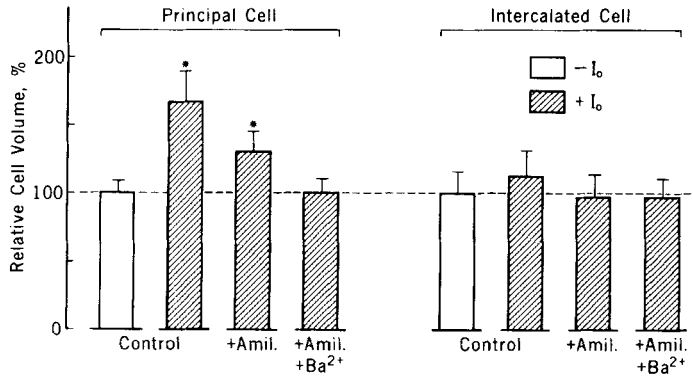

FIG. 3. Differential current-induced cell swelling properties of the principal and intercalated cells. Relative cell volume of each cell type was measured in the absence (open bars) or presence (matched bars) of a lumen-to-bath positive current pulse (150 nA, 10-sec duration). The influence of luminal addition of amiloride (50 μM) or amiloride (50 μM) plus barium (5 mM) is indicated. See text for details. $*P < 0.05$.

coupled. Furthermore, these studies demonstrate that the apical cell membrane of the principal cell possesses an amiloride-sensitive sodium conductance and barium-sensitive potassium conductance and, hence, that the principal cell is likely primarily responsible for sodium and potassium transport, a finding consistent with previous morphological data (Kaissling and Le Hir, 1982; Rastegar *et al.*, 1980; Stanton *et al.*, 1986; Wade *et al.*, 1979).

IV. CELLULAR ACTIONS OF MINERALOCORTICOIDS ON THE PRINCIPAL CELL

A. Apical Cell Membrane Potassium and Sodium Conductive Pathways

Along with the demonstration that the principal cell was primarily responsible for sodium and potassium transport in the cortical collecting duct, studies were undertaken to evaluate the influence of mineralocorticoids on the transport properties. In all studies, animals were used with intact adrenal glands where the plasma mineralocorticoid levels were varied chronically over several days, as indicated.

In the first series of studies, the influence of elevating plasma mineralocorticoid levels on the principal cell apical membrane sodium and potassium conductances was assessed as a function of time of treatment (Sansom and O'Neil, 1985). Rabbits were initially maintained on a high Na diet for 1 week or more to moderately suppress plasma aldosterone levels and then treated daily with the mineralocorticoid deoxycorticosterone acetate (DOCA, 2 mg/kg/day, intramuscularly) for 1–16 days. At the end of the specified treatment period, a tubule was isolated, perfused, and bathed in an NaCl Ringer's solution. Microelectrode techniques were applied to quantitate the apical cell membrane sodium and potassium conductances, as indicated by the amiloride-sensitive and barium-sensitive conductances, respectively, as outlined and validated previously (Sansom and O'Neil, 1985).

As summarized in Fig. 4A, after 1 day of mineralocorticoid treatment, the apical membrane sodium conductance increased from near 0.7 to 1.6 mS cm^{-2} and continued to increase to slightly higher levels near 2 mS cm^{-2} with prolonged treatment for 2 weeks. In contrast, the apical membrane potassium conductance, which normally is much greater than the sodium conductance, averaged near 4 ms cm^{-2} in control conditions and was not altered after 1 day of mineralocorticoid treatment. However, after 4 days of treatment, the potassium conductance doubled in value and remained elevated with continued treatment for 2 weeks. It would appear that the responses of the two apical membrane conductances to mineralocorticoids differ, with the changes in the sodium con-

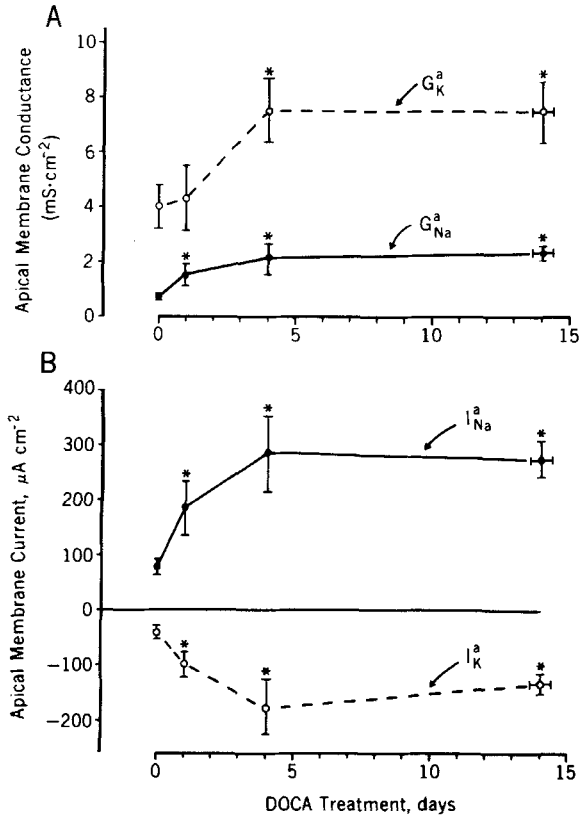

FIG. 4. Influence of mineralocorticoid treatment, DOCA (2 mg/kg/day). Effect on (A) the apical border sodium conductance, G^a_{Na}, and potassium conductance, G^a_K. (B) Effect on the apical border sodium current (sodium influx), I^a_{Na}, and potassium current (potassium efflux to lumen), I^a_K. [Adapted from Sansom and O'Neil (1985).] $*P < 0.05$.

ductance reflecting an "early response" and the changes in the potassium conductance reflecting a "late response."

The influence of the above mineralocorticoid treatment was extended to evaluate the influence on the sodium and potassium fluxes via their respective conductive pathways. In these studies, the tubules were perfused and bathed with an NaCl Ringer's solution without bicarbonate (and CO_2) so that the only active ionic fluxes across the epithelium were for sodium and potassium. As discussed in detail previously (Sansom and O'Neil, 1985), under these conditions the selective effects of amiloride and barium can be used to obtain an estimate of the apical membrane sodium current, I^a_{Na}, and potassium current, I^a_K, and associated electromotive forces. Within the first day of mineralocorticoid treatment, the

apical membrane sodium current doubles and continues to shift upward with prolonged tretment for up to 2 weeks, essentially mirroring the changes in sodium conductances. The apical membrane potassium current, which is normally negative, reflecting secretion of potassium, is likewise elevated after 1 day of treatment despite the fact that the conductance has not changed. This stimulation of K^+ secretion occurs as a result of two other changes. First, the increase in sodium conductance depolarizes the apical membrane, thereby favoring potassium secretion, and second, the increased transport turnover of the sodium:potassium exchange pump to accommodate the increased sodium load tends to elevate intracellular potassium activity, again favoring potassium secretion (see Sansom and O'Neil, 1985). The combined effect is to increase the net driving force for potassium secretion after 1 day of treatment, thereby elevating potassium secretion without a change in potassium conductance. With continued mineralocorticoid treatment for 4 days or more, the potassium current is elevated further, reflecting the increase in potassium conductance of the apical cell border.

B. Basolateral Cell Membrane Na^+,K^+-ATPase Activity

It is evident from the above reported data that mineralocorticoids in part exert their influence on sodium and potassium transport by regulating the apical cell membrane conductances to these ions. It is also known that mineralocorticoids may influence the sodium:potassium exchange pump at the basolateral border, since it has been reported by several laboratories that mineralocorticoids may influence the Na^+,K^+-ATPase activity levels in this segment of the nephron (see Katz, 1982). Hence, in a parallel series of studies, we evaluated the influence of mineralocorticoids on sodium pump levels. These levels were evaluted as the Na^+,K^+-ATPase activity of single nephron segments. The activity was measured using a microfluorometric assay evaluated under V_{max} conditions for the enzyme, providing a measure of the total amount of enzyme present (O'Neil and Dubinsky, 1984). Under these conditions, Na^+,K^+-ATPase activity is directly proportional to the number of ouabain binding sites (El Mernissi and Doucet, 1984a).

In initial studies, the influence of either suppressing endogenous mineralocorticoid levels via dietary alterations (O'Neil and Hayhurst, 1985b) or treatment with the aldosterone antagonist spironolactone (unpublished observation) was assessed. However, neither of these procedures applied for 1 week significantly reduced the Na^+,K^+-ATPase activity in the cortical collecting duct, indicating that more drastic methods, such as adrenalectomy, are required to suppress normal Na^+,K^+-ATPase activity. In contrast, elevation of either endogenous or exogenous plasma mineralocorticoid levels for 3–4 days markedly elevated

Na^+,K^+-ATPase activity. As shown in Fig. 5, elevation of exogenous mineralocorticoid levels for 3–4 days by treatment with DOCA resulted in an increase in Na^+,K^+-ATPase levels from near 15 pmol ADP min^{-1} mm^{-1} to near 31 pmol ADP min^{-1} mm^{-1}. Similarly, elevation of endogenous aldosterone levels for 3 days by maintaining the animals on a low sodium diet caused a similar increase in Na^+,K^+-ATPase levels. If, on the other hand, animals were treated with high doses of glucocorticoid dexamethasone (DEXA) for 3–4 days (0.5 mg/kg/day, subcutaneously), Na^+,K^+-ATPase activity was not altered from normal values. It is therefore evident that mineralocorticoids, and not glucocorticoids, are primarily responsible for regulating Na^+,K^+-ATPase and, hence, sodium and potassium transport, in cortical collecting duct cells of animals with intact adrenal glands.

The temporal aspects of mineralocorticoid treatment on Na^+,K^+-ATPase were assessed in a similar manner to that for conductive pathways, as discussed above. Animals were maintained on a high sodium diet for 1 week and then treated daily with DOCA (2 mg/kg/day, intramuscularly) (O'Neil and Hayhurst, 1985b). As shown by the data in Fig. 6, the Na^+,K^+-ATPase activity averaged near 16 pmol ADP min^{-1} mm^{-1} prior to treatment with mineralocorticoid and was not influenced significantly after 1 day of treatment. After 2 days or more of treatment, Na^+,K^+-ATPase activity doubled. Hence, in aldosterone-replete animals with intact adrenal glands, mineralocorticoids stimulate Na^+,K^+-ATPase activity, but only after a latent period of approximately 1 day.

Mujais *et al.* (1985) have also noted a long latent period in adrenalectomized

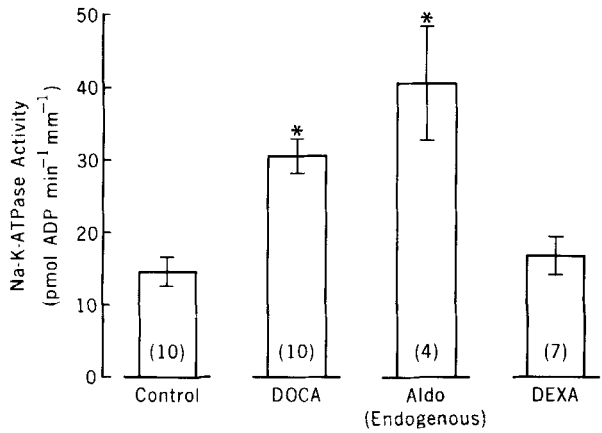

FIG. 5. Influence of steroids on the cortical collecting duct Na^+,K^+-ATPase activity. Animals were treated for 3–4 days with DOCA (2 mg/kg/day), dexamethasone, DEXA (0.5 mg/kg/day), or a low sodium diet to elevate endogenous plasma aldosterone levels (Aldo). $*P < 0.05$.

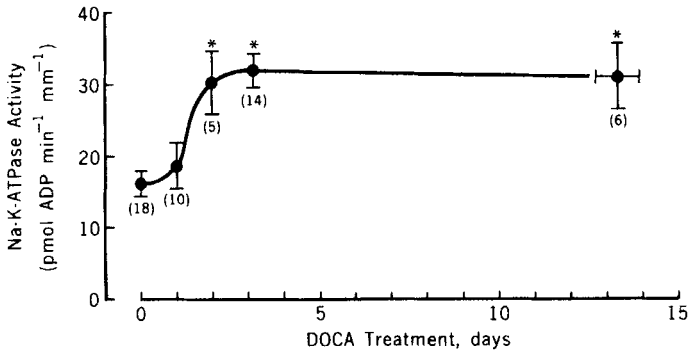

FIG. 6. Time course of the effects of mineralocorticoid treatment, DOCA (2 mg/kg/day), on the cortical collecting duct Na^+,K^+-ATPase activity. [From O'Neil and Hayhurst (1985b).] $^*P < 0.05$.

rats treated with aldosterone. Using osmotic minipumps for delivery of aldosterone so as to "clamp" plasma aldosterone concentration at defined levels, aldosterone treatment was found to cause a stimulation in the Na^+,K^+-ATPase of the collecting duct above initial control values within 12 hr. In aldosterone-depleted adrenalectomized animals where Na^+,K^+-ATPase levels of the cortical collecting duct were depressed by 70–80% from normal control values, aldosterone treatment for 3 hr has been reported in some studies (El Mernissi and Doucet, 1983; Horster *et al.*, 1980; Petty *et al.*, 1981), but not in others (Doucet and Katz, 1981; Mujais *et al.*, 1984), to increase Na^+,K^+-ATPase activity back to its prior control values. This response, if it is present, is likely too rapid to arise from increased synthesis of Na^+,K^+-ATPase subunits and, hence, may reflect activation of latent pump sites. In contrast, Na^+,K^+-ATPase activity above normal control levels after a latent period of 12–24 hr as reported by O'Neil and Hayhurst, (1985b) and by Mujais *et al.* (1985), would be consistent with increased synthesis of the enzyme. Specifically, Geering *et al.* (1982) demonstrated that aldosterone increases the rate of synthesis of the two subunits of Na^+,K^+-ATPase of the toad bladder, but only after a latent period of 6 hr. Since the subunits must still be packaged into an active Na^+,K^+-ATPase complex at the cell membrane, an additional delay would follow before an increase in Na^+,K^+-ATPase activity would be apparent. Similarly, Lo and Edelman (1976) have demonstrated that renal Na^+,K^+-ATPase normally has a slow rate of turnover and that stimulation of synthesis of proximal tubule Na^+,K^+-ATPase subunits by thyroid hormone is accompanied by an increase in Na^+,K^+-ATPase activity only after a latent period of approximately 12 hr. In view of these considerations, mineralocorticoid-induced Na^+,K^+-ATPase activity reported here and previously (O'Neil and Hayhurst, 1985b) would be most consistent with increased synthesis of the enzyme subunits.

C. Sodium-Dependent Modulation of Na^+,K^+-ATPase

It is apparent from the above data that mineralocorticoids have multiple actions on the cortical collecting duct. The initial action (early response) is to increase the sodium conductance of the apical cell membrane prior to any effect on either apical membrane potassium conductance or basolateral membrane Na^+,K^+-ATPase activity (see summary data, Fig. 7). It is also apparent that the late responses consisting of increased potassium conductance and Na^+,K^+-ATPase activity closely parallel each other both temporally and stoichiometrically so that these two parameters may share a common control mechanism or synthetic pathway. Nonetheless, in view of the temporal separation of the early and late responses, it may be that the increases in potassium conductance and Na^+,K^+-ATPase are dependent upon the increases in sodium conductance and sodium influx into the cell. This notion was directly examined for the response of Na^+,K^+-ATPase activity using the Na channel blocker amiloride as a probe.

Rabbits were maintained on a high Na, low K diet for 1 week prior to study so as to reduce possible potassium loading of the animal during amiloride treatment. At the end of this period, animals were treated for 3–4 days with DOCA alone, as described above, with DOCA and amiloride (10 mg/kg/twice daily, subcutaneously), or with amiloride alone. As summarized in Fig. 8, DOCA treatment caused the usual stimulation in Na^+,K^+-ATPase activity, but this was markedly blunted by simultaneous treatment with amiloride. Treating the animals with amiloride alone had no influence on the activity. It is likely, therefore, that sodium entry modulates mineralocorticoid-stimulated Na^+,K^+-ATPase activity only.

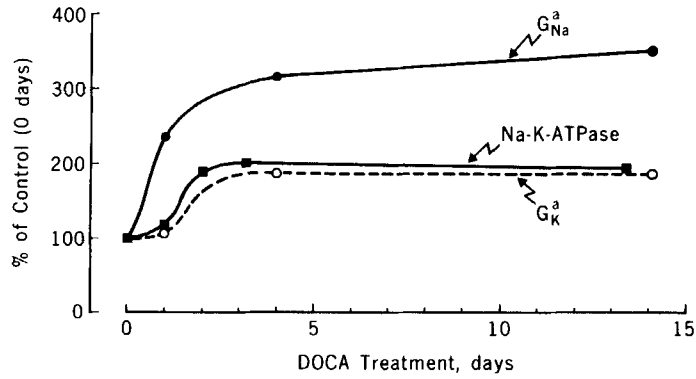

FIG. 7. Comparison of the time-dependent effects of mineralocorticoid treatment on the cortical collecting duct Na^+,K^+-ATPase activity and the apical cell membrane partial conductances, G^a_{Na} and G^a_K. Data from Figs. 4 and 6.

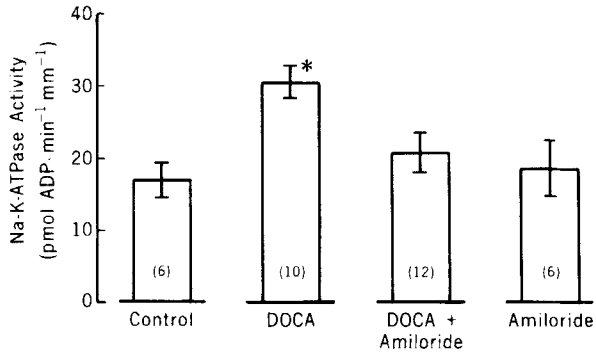

FIG. 8. Influence of DOCA and the sodium channel blocker amiloride on Na^+,K^+-ATPase activity. Animals were treated for 3–4 days with DOCA (2 mg/kg/day), amiloride (10 mg/kg/twice daily, subcutaneously), or DOCA and amiloride. [Data from O'Neil and Hayhurst (1985b).] $*P < 0.05$.

The results of recent studies on the effects of a low Na diet and endogenous aldosterone are likewise consistent with Na-dependent modulation of Na^+,K^+-ATPase (O'Neil and Hayhurst, 1985b). When rabbits were placed on a low Na diet, serum aldosterone levels were observed to rise from 16 to near 70 ng/dl after 3–4 days, and then continue to trend upward with prolonged maintenance on the low Na diet for over 2 weeks, reflecting both sodium and volume depletion. As the animal became volume depleted, a greater fraction of the filtered sodium load would be expected to be reabsorbed by the proximal tubule. In turn, less sodium would be delivered to the cortical collecting duct, resulting in reduced influx of sodium into the epithelial cells within several days (see O'Neil and Hayhurst, 1985b). Indeed, Na^+,K^+-ATPase activity of cortical collecting duct isolated from these animals was elevated by 2- to 3-fold after 3–4 days, but then slowly declined, returning to the initial control values after 2 weeks of treatment. It is apparent that under these conditions as well, the aldosterone-induced increase in Na^+,K^+-ATPase is modulated by sodium entry into the cell. Hence, the combined demonstration of an early response of the apical membrane sodium conductance to mineralocorticoids and the amiloride and sodium sensitivity of the mineralocorticoid-induced Na^+,K^+-ATPase activity provide convincing evidence that the mineralocorticoid-induced Na^+,K^+-ATPase activity is modulated by sodium entry into the cell.

Additional support for a role of sodium in modulating Na^+,K^+-ATPase activity has been reported by others. Petty *et al.* (1981) demonstrated that in adrenalectomized rabbits, stimulation of Na^+,K^+-ATPase activity of the cortical collecting duct by aldosterone (within 3 hr) could be blocked by treatment with amiloride. Similarly, Handler and co-workers (Handler *et al.*, 1981) noted that in the aldosterone-sensitive A6 cell line, amiloride abolished an aldosterone-

induced increase in the number of ouabain binding sites. Sodium entry into these cells must be modulating the mineralocorticoid-induced active pump sites.

The site at which sodium entry may be modulating Na^+,K^+-ATPase is not known. Geering *et al.* (1982) observed that aldosterone-induced increase in the synthesis of the two subunits of Na^+,K^+-ATPase of the toad urinary bladder was not influenced by blocking sodium entry into the cells with amiloride. The site of the sodium-dependent modulation must therefore be at some posttranslational site after synthesis, e.g., glycosylation, phosphorylation, packaging of subunits into cytoplasmic vesicles, or exocytosis. There is circumstantial evidence that an exocytic insertion step for the sodium pump may be a site of modulation. Williams (1975) and Kanno *et al.* (1977) have shown that enzyme secretion and, hence, exocytic processes, in pancreatic acinar cells is dependent upon sodium entry into the cell. This effect has been shown to be related to the influence of sodium entry on intracellular calcium and hence not a direct effect of sodium (Cochrane *et al.*, 1975; Poulsen and Williams, 1977). More recently, Hudson and Schultz (1984) and Lau *et al.* (1984) reported that stimulation of sodium entry into the *Necturus* small intestinal cells was characterized by an initial transient rise in intracellular sodium activity. This was followed by a parallel increase in the short-circuit current (net sodium flux) and basolateral membrane barium-sensitive potassium conductance at a time when the intracellular sodium activity had decreased back to its initial control value. The elevated short-circuit current and potassium conductance must therefore reflect recruitment or activation of additional sodium pumps and potassium leak units into the basolateral membrane. Lewis *et al.* (1985) also demonstrated that conditions which stimulate sodium transport in the toad urinary bladder were accompanied by a parallel increase in the sodium pump current and basolateral membrane conductance. Furthermore, these changes in the basolateral membrane conductance could be mimicked by cell swelling alone (Lewis *et al.*, 1985; Lau *et al.*, 1984), implying that sodium entry may be exerting its effects indirectly as a consequence of either cell swelling or alteration in some other parameter such as cell calcium activity. In view of these considerations, it may be that sodium-dependent exocytic mechanisms are in part responsible for both the acute regulation in sodium transport as well as for the more chronic changes in transport, which may account for sodium-dependent modulation of mineralocorticoid-induced Na^+,K^+-ATPase activity reported for the cortical collecting duct.

D. Relation between Basolateral Membrane Area and Na^+,K^+-ATPase

The basolateral membrane of the cortical collecting duct cells appears to be under the control of mineralocorticoids. It was shown by Wade *et al.* (1979) that

treatment of rabbits with mineralocorticoids causes a marked amplification in the basolateral cell membrane of the principal cell only. This selective influence of mineralocorticoids on the principal cell has been noted in both rabbit and rat (Kaissling and Le Hir, 1982; Rastegar *et al.*, 1980; Stanton *et al.*, 1986) and provides additional evidence that the principal cell is primarily responsible lor sodium and potassium transport, as noted above. Interestingly, changes in the basolateral membrane area are closely paralleled by changes in Na^+,K^+-ATPase activity of the tubule (Kaissling and Le Hir, 1982; Le Hir *et al.*, 1982b), as would be expected if the Na^+,K^+-ATPase enzyme were inserted into the basolateral membrane via an exocytic process, thereby adding enzyme and membrane together. This close parallelism between enzyme and membrane is not likely restricted to the cortical collecting duct, as a close association appears to exist between these two parameters for all nephron segments. As shown by a plot of these two parameters in Fig. 9 for all rabbit nephron segments in which data already exist, the basolateral membrane area and Na^+,K^+-ATPase activity are closely correlated ($r = 0.981$) over a wide range of values. It would appear that these two parameters are obligatorily coupled. This is particularly evident for the effects of mineralocorticoids on the rabbit cortical collecting duct. Using our own data in which animals were similarly treated, chronic DOCA treatment was observed to cause a doubling in both Na^+,K^+-ATPase activity (Fig. 6) and absolute basolateral membrane area (Wade *et al.*, 1979), as shown in Fig. 9 (points 6a and 6b). These alterations correspond to the more global relation existing among all nephron segments (Fig. 9).

The close parallelism between basolateral membrane area and Na^+,K^+-ATPase activity leads to several conclusions. First, since elevation of Na^+,K^+-ATPase activity is accompanied by a parallel increase in basolateral membrane area, Na^+,K^+-ATPase activity must largely reflect incorporation of active enzyme at the basolateral membrane and not of latent pump sites within the cytoplasm. Second, chronic regulation of basolateral membrane Na^+,K^+-ATPase activity must occur by simple insertion (synthesis) and removal of pump sites via exocytic/endocytic mechanisms—thereby accounting for basolateral membrane area changes—and not by activation/deactivation of latent pump sites within the membrane. This view is directly supported by the demonstration of Mujais *et al.* (1985) and El Mernissi and Doucet (1984b) that mineralocorticoid treatment causes a doubling in ouabain binding sites in the cortical collecting duct, again paralleling changes in Na^+,K^+-ATPase activity. Third, if inserting of active pumps and membrane occurs simultaneously via an exocytic process, removal of pump sites from the membrane would be expected to occur via an endocytic process, similar to that reported for HeLa cells (Pollack *et al.*, 1981), thereby providing a pathway for membrane and pump recycling or lysosomal degradation. Finally, since alterations in Na^+,K^+-ATPase activity among nephron segments tend to parallel alterations in net sodium transport among these

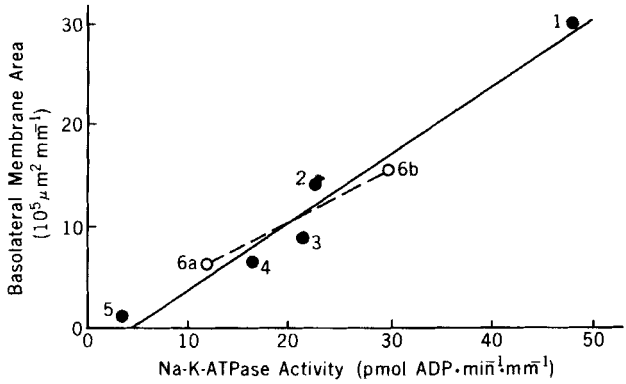

FIG. 9. Relationship between Na^+,K^+-ATPase activity and absolute basolateral membrane area of rabbit nephron segments. Nephron segments are (1) proximal convoluted tubule (S_1), (2) proximal straight tubule (S_2), (3) cortical thick ascending limb, (4) cortical collecting duct, control, (5) thin descending limb, (6a and 6b), cortical collecting duct values for control and DOCA-treated animals, respectively, of similarly treated animals from O'Neil and associates (O'Neil and Dubinsky, 1984; Wade *et al.*, 1979) (open circles). Closed circles are average values from the literature. The Na^+,K^+-ATPase activities were from Barlet *et al.* (1985), Garg *et al.* (1981), Katz *et al.* (1979), O'Neil and Dubinsky (1984), and Schmidt and Horster (1978). The basolateral membrane areas (except thin descending limb) were from Kaissling and Le Hir (1982) (after correction for tubule length using tubule outer diameter of 27 μm), Welling *et al.* (1978, 1981), Welling and Welling (1975, 1976), and Wade *et al.* (1979). The basolateral membrane area of the thin descending limb was determined in the present study, as described previously (Wade *et al.*, 1979), and averaged 1.1 ± 0.1 × 10^5 μm^2 mm^{-1} tubule length (n = 6). Micrographs were kindly provided by K. Karnaky and L. Garretson. The best fit linear regression equation of the average values (closed circles) is Basolateral membrane area = 0.67 × 0.08 (Na^+,K^+-ATPase activity) − 3.2 ± 2.0 (r = 0.981)

segments (Garg *et al.*, 1981), regulation of Na^+,K^+-ATPase activity and basolateral membrane area may reflect sodium-dependent modulation of these parameters in a manner similar to that heretofore mentioned for the cortical collecting duct.

V. SUMMARY

The mammalian cortical collecting duct avidly reabsorbs sodium and secretes potassium. It has been shown in this and earlier studies that the principal cell of this nephron segment is primarily responsible for the transport of both cations and that this cell is a mineralocorticoid target site. Mineralocorticoids exert multiple effects on this cell type to bring about chronic regulation of sodium and potassium transport and the maintenance of electrolyte balance.

The mechanisms of action by which mineralocorticoids exert their influence

on sodium and potassium transport by the principal cell are depicted in Fig. 10. The initiating events have been studied in detail by several laboratories (see Edelman, 1981; Marver and Kokko, 1983) and are summarized in the figure. A mineralocorticoid hormone such as aldosterone (A) diffuses into the cell and binds to a cytoplasmic receptor (R), forming a complex (RA) which migrates to the nucleus via a temperature-dependent process. The complex binds to a receptor site on chromatin, inducing transcription and the synthesis of new messenger RNA and, in turn, the production of a myriad of new aldosterone-induced proteins. All physiological actions of mineralocorticoids are dependent on protein synthesis, since blocking protein synthesis with actinomycin D abolishes the effects of mineralocorticoids on sodium and potassium transport.

After these initial events have been established, which require ~ 1 hr, the physiological effects are expressed. As shown in the present study and others (Sansom and O'Neil, 1985), the apical cell membrane sodium conductance is increased, first resulting in increased sodium entry from the lumen and, hence, in net sodium transport by the cell. The increased conductance likely reflects activation of preexisting sodium channels at the apical cell membrane, as shown for the toad urinary bladder (Palmer *et al.*, 1982), and not the synthesis of new channels (Garty and Edelman, 1983). The change in the apical membrane sodium conductance occurs prior to any change in other parameters and therefore reflects an early response to mineralocorticoids, similar to that observed for the toad urinary bladder.

While not depicted in Fig. 10, the initial increase in the apical membrane sodium conductance and sodium entry will depolarize the apical cell membrane,

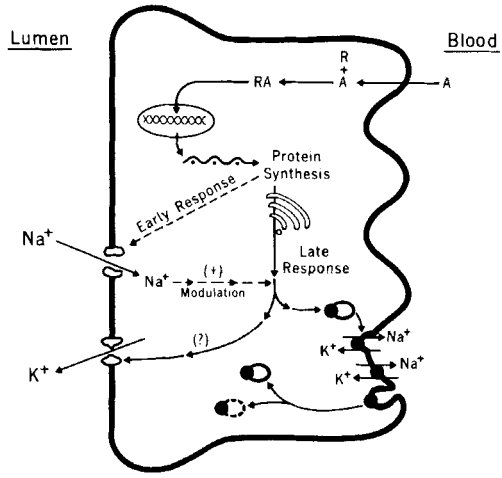

FIG. 10. Cellular model of mineralocorticoid actions in the principal cell of the cortical collecting duct. See text for details.

thereby creating a favorable driving force for potassium secretion into the lumen. Furthermore, the increased rate of sodium transport will result in an increased turnover transport rate of the already existing sodium pumps, resulting in increased potassium uptake from the peritubular (blood) side of the tissue. The combined net effect of depolarizing the apical membrane and increasing uptake of potassium across the basolateral border is to create a more favorable driving force for potassium secretion into the lumen. Hence, if a potassium channel is present at the apical cell membrane, as it normally is, potassium secretion will be stimulated during this early response without a change in the potassium conductance.

In addition to the early response, there is a delayed or late response that is expressed after a latent period of 12–24 hr, at least in adrenal-intact, aldosterone-replete animals. This late response is characterized by a parallel increase in basolateral membrane Na^+,K^+-ATPase activity and apical membrane potassium conductance. Since the renal Na^+,K^+-ATPase turnover is slow (Lo and Edelman, 1976) and aldosterone has been shown to induce synthesis of both Na^+,K^+-ATPase subunits in the toad urinary bladder (Geering *et al.*, 1982), the late mineralocorticoid-induced increase in Na^+,K^+-ATPase activity of the cortical collecting duct likely reflects increased synthesis of the enzyme. The newly synthesized subunits would be processed through the Golgi apparatus, packaged into cytoplasmic vesicles, sorted, and inserted into the basolateral membrane, thereby adding both new pumps and membrane. The increase in the apical membrane potassium conductance parallels the increase in Na^+,K^+-ATPase, implicating a common regulatory site or pathway, and hence may also reflect increased synthesis of a potassium channel, although this view remains speculative. In any event, it is this late response, at least for Na^+,K^+-ATPase, that is modulated by sodium entry; recent evidence indicates that potassium conductance may likewise by modulated (Sansom *et al.*, 1985). Sodium entry acts as a positive modulator of this late response, so that with elevated sodium influx, mineralocorticoid-induced Na^+,K^+-ATPase activity is expressed, whereas with reduced sodium influx the activity is depressed. This modulation likely occurs at a posttranslational site, possibly at the final exocytic insertion step, although this remains to be directly assessed. While the mechanism of this regulation is unknown, it is evident that sodium entry serves as a physiological modulator by indirectly regulating the extent to which the cell can chronically adapt to the mineralocorticoid hormone. Hence, sodium entry plays a pivotal role in modulating the long-term transport capacity of the tubule both to reabsorb sodium and to secrete potassium.

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Chapter 10

Potassium and Acid–Base Balance

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Potassium and hydrogen ions undergo interactions in transport at both the cellular and renal levels, which appear to be crucial for the maintenance of normal homeostasis and which under certain conditions can lead to disordered regulation of either acid–base or potassium metabolism.

It is widely recognized that changes in systemic hydrogen ion concentrations are accompanied by alterations in cellular potassium content (2,58) and, conversely, that changes in potassium homeostasis can lead to alterations in intracellular pH (57). At the renal level, perturbations in systemic pH modify potassium transport and alterations in potassium influence acid–base regulation (1). Finally, chronic disorders of acid–base metabolism can be accompanied by abnormalities in K^+ homeostasis (19) and vice versa.

There are a number of excellent reviews dealing with these topics, and an attempt will not be made here to cover all these aspects of the potassium–hydrogen ion interrelationship. Rather, the focus will be on the effect of alterations in potassium homeostasis on renal hydrogen ion handling, and this information will be formulated into a postulate to explain how this interaction functions to maintain K^+ and H^+ homeostasis under normal conditions and how it can result in disturbances in acid–base regulation in response to chronic perturbations in K^+ balance.

The regulation of hydrogen ion homeostasis by the kidney is dependent upon several discrete, but integrated processes, which include the following: bicarbonate transport by the proximal nephron; an H^+ and probably also an HCO_3 secretory mechanism at distal nephron sites, including the distal convoluted

tubule, and the cortical and medullary collecting duct; and the capacity of the kidney to produce the major urinary buffer, ammonia. (64).

I. POTASSIUM AND RENAL AMMONIA PRODUCTION

A large body of evidence exists to support an intimate relationship between potassium homeostasis and renal ammonia production (63). My interest in this topic was kindled initially by a study of urine acidification in normal men with mild (~200 mEq deficit), experimentally induced potassium depletion (62). As shown in Fig. 1, in response to an acute NH_4Cl load, urine pH was elevated in the potassium-depleted state in comparison with paired observations under conditions of normal potassium balance. The increase in urine pH was accompanied by an increase in both ammonium and net acid excretion, shown in Fig. 2, and also by an increase in the concentration of free ammonia in the urine. This combination of events was best explained by a K^+ depletion-induced primary

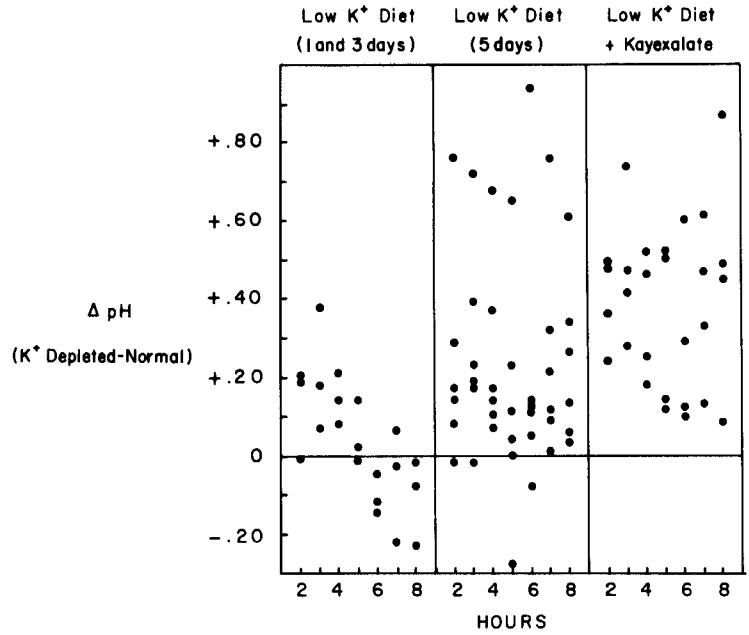

FIG. 1. The effect of potassium depletion on urine pH. Each point represents a comparison between the response to ingestion of oral NH_4Cl (at 0 hours) for a paired potassium-depleted and normal study in normal human volunteers. Potassium depletion of sufficient magnitude significantly increased urine pH. (Reproduced from *The Journal of Clinical Investigation*, 1970, **49**, 813 by copyright permission of The American Society for Clinical Investigation.)

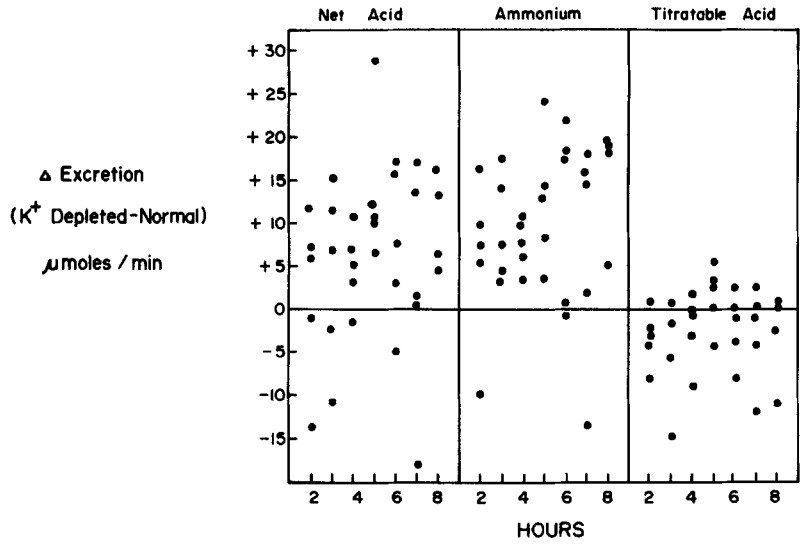

FIG. 2. Response of urinary acid–base parameters to potassium depletion. The increase in urine pH shown in Fig. 1 is accompanied by an increase in both net acid and ammonium excretion. (Reproduced from *The Journal of Clinical Investigation*, 1970, **49**, 813 by copyright permission of The American Society for Clinical Investigation.)

increase in ammonia diffusion into the urine, resulting either from a decrease in renal blood flow (thereby causing a greater percentage of the ammonia produced to be trapped in the urine) or from an increase in cellular ammonia production.

When similar studies were carried out in normal human volunteers ingesting a high potassium diet (3–6 mmol/kg/day) for 5 days, the results were the exact converse of those found with mild K^+ depletion (70). Urine pH was lower than with a normal dietary potassium, and this was accompanied by a decrease in both ammonium and net acid excretion. Thus, a high potassium diet appeared to alter urinary acidification either by increasing renal blood flow or by diminishing renal ammonia production.

A substantive body of evidence demonstrates that K^+ depletion *in vivo* stimulates renal ammonia production. Direct measurements of renal ammonia production in intact humans, dogs, and rats have shown increased ammoniagenesis under conditions of potassium depletion (3,16,73). This capacity for increased ammonia production is demonstrable when either renal cortical slices or mitochondria from K^+-depleted animals are investigated *in vitro* (Fig. 3), indicating the existence of an adaptation comparable to that which accompanies chronic metabolic acidosis (34,46,63,66,67). The precise metabolic mechanism accounting for the adaptive response to K^+ depletion has not been delineated, but, as

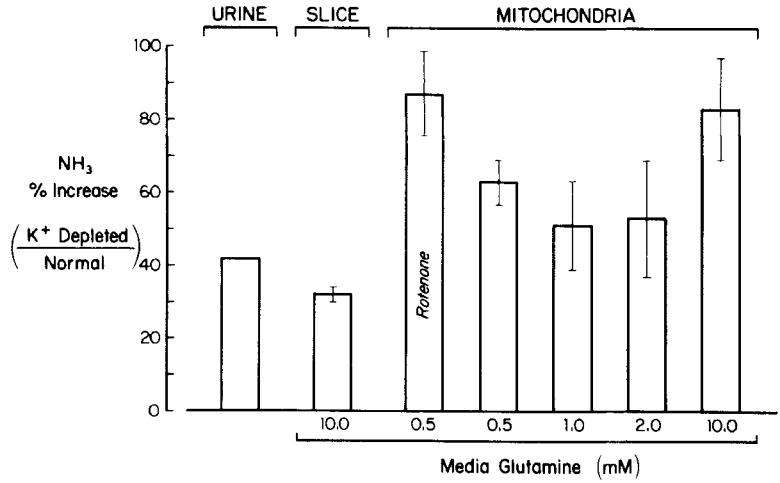

FIG. 3. Comparative changes in urinary, renal cortical slice and mitochondrial NH_3 metabolism during K^+ depletion. These data were obtained using rats placed on a low potassium diet for 4 weeks. K^+ depletion results in an adaptive increase in the capacity for NH_3 production by both renal cortical tissue and isolated mitochondria. (Reprinted with permission from Ref. 66.)

shown in Table I, all studies to date have yielded metabolic patterns virtually identical to those found with adaptation to chronic metabolic acidosis (63). Both conditions might enhance NH_3 production as a result of a decrease in intracellular pH, or alternatively, they might both activate other common or different effectors that modify the metabolic cascade in a similar fashion.

In addition to our studies in humans, a number of other observations in humans, rats, and dogs have documented a concurrent decrease in urine pH and ammonium excretion in response to a high potassium intake (23,25,27,32,44,55,61,70). While these studies are all consistent with a high K^+-induced decrease in ammoniagenesis, direct measurements of renal ammonia production *in vivo* have not been reported.

A few studies have examined the effects of high potassium on ammonia production by renal tissue *in vitro* (53,56,66,67). Ingestion of a high potassium diet had no effect on renal ammonia production by either the isolated perfused rat kidney or isolated renal cortical mitochondria (53,67). By contrast, a modest 5% decline in ammoniagenesis was found with renal cortical slices incubated *in vitro* and a 32% decrease with outer medullary slices (66). The reason for the discrepant findings with different *in vitro* techniques is unclear; however, the isolated perfused kidney appears to mimic ammoniagenesis *in vivo* more reliably than cortical slices (68). Thus, from the evidence available currently, it appears unlikely that a high K^+ diet results in an adaptive down-regulation in the renal capacity to produce ammonia.

TABLE I

METABOLIC CHANGES IDENTIFIED WITH POTASSIUM DEPLETION IN RATS COMPARED TO THOSE FOUND WITH METABOLIC ACIDOSIS[a–c]

Metabolic pathway	Potassium depletion	Metabolic acidosis
Cytosol		
1. Transaminase (glutaminase II) enzyme activity	↑	↑
2. γ-glutamyltransferase and/or transpeptidase (phosphate-independent glutaminase)		
a. Enzyme activity	—	NC
b. NH_3 production	—	NC
3. Glutamine synthetase, glutamine production from glutamate	↓	↓
4. Purine nucleotide cycle enzyme activity	↑	↑
Mitochondria		
5. Phosphate-dependent glutaminase pathway		
a. Mitochondrial NH_3 production	↑	↑
b. Glutamine entry step	↑	↑
c. Deamidation		
i. PDG activity	↑	↑
ii. Mitochondrial NH_3 production with rotenone	↑	↑
d. Deamination NH_3 production glutamate (intact tissue)	↑	↑
e. TCA cycle		
i. $^{14}CO_2$ production from [U-^{14}C]Gln (slices)	↑	↑
ii. $^{14}CO_2$ production from [U-^{14}C]Gln (mitochondria)	↑↓	↑↓
iii. Citrate utilization	↑	↑
iv. Malate exit	—	↑
f. Glucose production[d]		
i. PEPCK activity	↑	↑
ii. Glucose production glutamine	↑	↑

[a] Reprinted from *Kidney International* (Vol 11:453–465, 1977), with permission.

[b] The symbols are ↑, increase; ↓, decrease; NC, unchanged; —, undescribed.

[c] Both potassium depletion and metabolic acidosis refer solely to events in the rat.

[d] This process is initiated in the mitochondrion and completed in the cytosol.

By contrast, recent studies using the isolated perfused kidney, renal cortical tubules, and renal cortical slices all indicate that a high potassium concentration can directly inhibit renal ammonia production (53,56). This suppressive effect has been demonstrated with K^+ concentrations in the range of 7.5–10.4 m*M* (53). The precise site of metabolic inhibition is controversial; some data suggest inhibition of the glutamate dehydrogenase step, while other studies point toward a primary effect on either glutamine transport into the mitochondria and/or on phosphate-dependent glutaminase (53,56).

The direct effect of a low K^+ concentration on ammonia production has also been investigated. We found that 2.0 m*M* K^+ did not stimulate NH_3 production

TABLE II
EFFECT OF K^+ ON RENAL H^+ TRANSPORT

Activity	Chronic K^+ depletion	Ingestion high K^+ diet	Hypokalemia	Hyperkalemia
NH_3 production	↑	No effect	No effect	↓
Distal nephron H^+ transport	↑	?↓	No effect	No effect
Proximal HCO_3 reabsorption	↑	?	No effect	?↓

by either rat isolated perfused kidney or renal cortical tubules (53). Sleeper and co-workers found a modest (10%) stimulation of ammoniagenesis when renal cortical slices were incubated in a K^+-free media (56). However, these latter studies are difficult to interpret in view of the problems inherent in the slice methodology and the unphysiological level of K^+ employed.

To summarize, as depicted in Table II, there is convincing evidence that K^+ depletion *in vivo* stimulates and that a high K^+ suppresses renal ammonia production. However, the mechanisms appear to differ, since the low K^+ effect is only clearly demonstrable with depletion *in vivo* and not with manipulations of potassium concentration *in vitro* while the high K^+ effect is demonstrable in response to direct *in vitro* changes in K^+ concentration and does not appear to be an adaptive phenomenon induced *in vivo*.

Investigation has also been carried out in both humans and rats to determine whether changes in ammonia production have any effect on potassium excretion. As shown in Fig. 4, increased ammonia production, which can be induced without altering systemic acid–base status by increasing the availability of the ammoniagenic substrate glutamine, results in enhanced ammonium excretion and a concurrent decrease in potassium excretion (29,52,69). This reciprocal relationship between ammonia and potassium has been demonstrated in humans, in intact rats, and with the isolated rat kidney perfused *in vitro* (29,52,69). With the isolated perfused kidney, the potassium response parallels ammonium excretion rather than production, indicating that the potassium sparing effect is a direct result of the increase in urinary ammonium excretion (52). Micropuncture studies in the rat indicate that the ammonium-induced decrease in K^+ secretion occurs at a site beyond the superficial late distal convoluted tubule, most likely the collecting duct (29). However, additional studies are needed to specifically implicate the collecting duct and to delineate the precise mechanism whereby an increase in ammonium excretion results in a decrease in potassium excretion. Although the quantitative impact of this phenomenon on K^+ excretion *in vivo* has not been resolved, studies with the isolated perfused kidney indicate that a change in ammonium excretion can reduce potassium excretion by ~ 50%, suggesting that this may be an important mechanism for K^+ regulation (52).

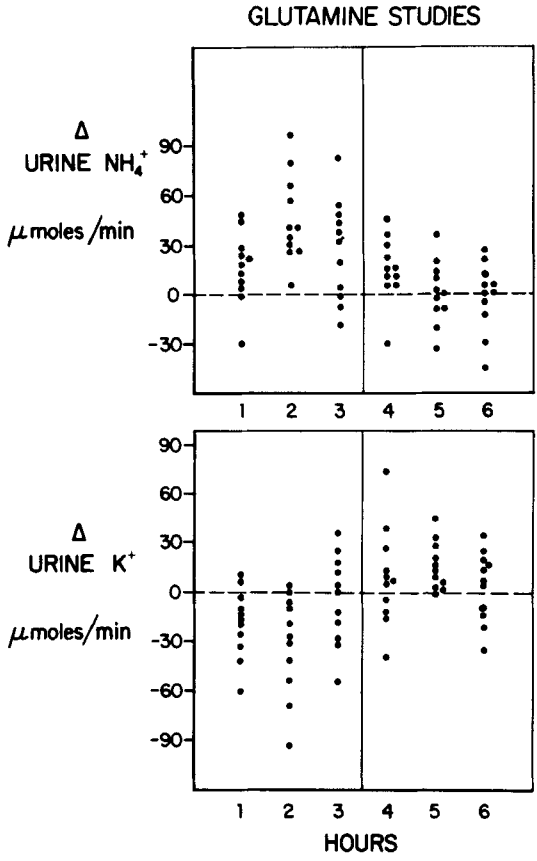

FIG. 4. Effect of increased ammonia production on potassium excretion. Urinary NH_4 excretion is increased significantly for the first 3 hr following glutamine ingestion when compared with an identical paired study performed in the absence of glutamine. This increase in NH_4 excretion is accompanied by a significant decrease in K^+ excretion, which reached maximal effect at 2 hr concomitant with the peak in NH_4 excretion. [Reprinted from *Kidney International* (Vol. II:453–465, 1977), with permission.]

II. EFFECT OF POTASSIUM ON THE DISTAL NEPHRON H^+ SECRETORY CAPACITY

In addition to modifying distal nephron H^+ by altering the availability of the urinary buffer ammonia, potassium may directly or indirectly modulate H^+ transport by the distal nephron epithelium via other mechanisms. For example, although an increased urinary pH with K^+ depletion and decreased pH with K^+

loading can be explained by alterations in ammonia production, concurrent specific K^+-induced alterations in H^+ transport cannot be discounted.

To date, this question has only been examined with indirect experimental techniques. Using U-B P_{CO_2} as a reflection of distal nephron H^+ secretion in intact animals, it has been concluded that K^+ depletion impairs proton secretion by the rabbit collecting duct, but also that hyperkalemia inhibits H^+ secretion by the distal nephron in the dog (20,47).

Recently, techniques were developed in our laboratory to indirectly measure both the maximal gradient and the H^+ secretory capacity of the distal nephron using the isolated perfused rat kidney (38,71). To measure the pH gradient, kidneys perfused without ammoniagenic substrate are subjected to progressively lower bicarbonate concentrations until the pH gradient between urine and perfusate is maximized, achieved at a perfusate pH less than 6.8. With this technique, no alteration in the maximal pH gradient was found with kidneys from rats subjected to ingestion of a K^+-free diet for 3 weeks or ingestion of a high potassium diet for 1–2 weeks (71).

To determine the H^+ secretory capacity of the distal nephron, kidneys are perfused with a low bicarbonate concentration to minimize bicarbonate delivery to the distal nephron and without glutamine in order to constrain ammonia production. Under these conditions, titration of the exogenously added buffer creatinine below pH 6.0 (called T. A. pH 6.0) is an indication of H^+ secretion at a functional distal nephron site (38). Progressive increases in perfusate creatinine are employed in these studies in order to provide sufficient buffer to saturate the distal nephron H^+ secretory capacity. Studies with kidneys from adrenalectomized rats with amiloride in the perfusate have documented that titration of creatinine below 6.0 represents H^+ secretion at a distal nephron site (37). Further validation of this technique is provided by the detection of alterations in distal nephron H^+ secretory capacity in response to chronic acid–base manipulation identical to those found with isolated collecting ducts perfused *in vitro* (38).

As shown in Fig. 5, using this technique, kidneys from rats on a K^+-free diet for 1 week exhibited a significantly increased distal nephron H^+ secretory capacity in comparison with kidneys from rats ingesting a high K^+ diet for 1 week. (36). The H^+ secretory capacity of controls fell between the low and high K^+ studies, but neither K^+ group exhibited a statistically significant difference from the controls. However, since the K^+-depleted kidneys exhibited an increase in H^+ secretory capacity similar to that found with chronic metabolic acidosis, it seems likely that K^+ depletion stimulates distal nephron H^+ secretion. Whether a high K^+ diet results in a decrease is more problematic. Of further interest, the difference in H^+ secretory capacity between the low and high K^+ kidneys persisted in the presence of amiloride, suggesting that the altered H^+ transport occurs by a mechanism independent of sodium-mediated (i.e., voltage-dependent) distal nephron H^+ secretion.

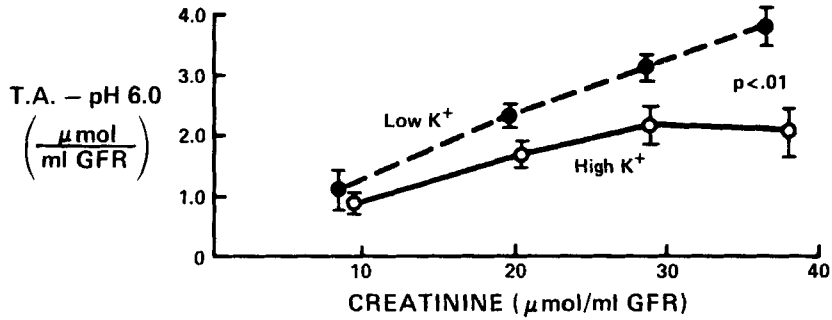

FIG. 5. Effect of K^+ depletion on the H^+ secretory capacity of the distal nephron. Kidneys from rats on a K^+-free diet for 1 week (low K^+) or high K^+ diet were perfused *in vitro* with a low HCO_3 perfusate which lacked glutamine. Creatinine was added to the perfusate in stepwise fashion to provide a urinary buffer. Under these conditions, titratable acid below pH 6.0 (T.A. pH 6.0) reflects H^+ secretion by a functional segment of the distal nephron. The significant increase in this parameter suggests that the H^+ secretory capacity of the distal nephron is increased by potassium depletion. (Reprinted with permission from Ref. 36.)

Several other observations lend support to the possibility that K^+ depletion stimulates H^+ secretion by the distal nehpron. Recent micropuncture studies reported in preliminary fashion indicate that the distal convoluted tubule reabsorbs HCO_3 against a steeper gradient during K^+ depletion (10). Furthermore, morphological studies indicate that the intercalated cells in the rat medullary collecting duct exhibit an increase in luminal membrane surface area under conditions of K^+ depletion (59). These mitochondria-rich, carbonic anhydrase-containing cells are believed to account for proton secretion, and an increase in their luminal surface area could reflect the incorporation of additional proton secretory units in their luminal membrane (22,43). While these data suggest that K^+ depletion may increase the H^+ transport capacity at distal nephron sites, further direct investigation of this issue is clearly required. It should also be emphasized that these studies have all been carried out with prolonged (i.e., 1 week or more) K^+ depletion and are not necessarily applicable to the physiological response to a decrease in K^+ intake.

In contrast to chronic alterations of K^+ homeostasis *in vivo, in vitro* manipulation of the ambient K^+ environment appears to have no effect on distal nephron H^+ transport. In recent studies, Jacobson found no alteration in HCO_3 reabsorption by the isolated rabbit medullary collecting duct in response to either an increase or a decrease in bath K^+ concentration (28).

Thus, as summarized in Table II, chronic K^+ depletion appears to increase H^+ secretion by the distal nephron, while the effect of chronic ingestion of a high K^+ diet is unresolved. To date, there is no evidence that changes in

potassium concentration per se directly modify proton transport by the distal nephron.

Finally, *in vivo* manipulation of K^+ homeostasis may also alter distal nephron H^+ secretion indirectly as a result of K^+-induced modulation of aldosterone secretion. Aldosterone can modify H^+ transport by the medullary collecting duct via a direct effect on the H^+ pump (60) and can potentially alter H^+ transport by the cortical collecting duct indirectly by altering Na-dependent (i.e., voltage-dependent) H^+ transport (37). Hypokalemia depresses, while hyperkalemia stimulates aldosterone secretion. Thus, aldosterone-mediated effects would tend to cause changes in H^+ secretion opposite to those which accompany prolonged modifications of K^+ homeostasis *in vivo*, especially K^+ depletion; however, it is not clear which stimulus might predominate under specific *in vivo* circumstances.

This discussion has focused solely on H^+ secretion by the distal nephron. It is now clear that the collecting duct can secrete bicarbonate as well as protons and that net H^+ secretion represents the difference between these two counteracting effects (45). To my knowledge, the influence of K^+ on the HCO_3 secretory pathway has not been evaluated.

III. EFFECT OF K^+ ON PROXIMAL HCO_3 REABSORPTION

A variety of studies *in vivo* have shown that K^+ depletion increases the T_m for bicarbonate reabsorption and that it is suppressed by hyperkalemia (15,21,40,48,50,51). Indeed, it has long been recognized that acute administration of KCl can result in an alkali diuresis (5,33,50). Surprisingly, there are few studies that have directly examined the effect of K^+ on the proximal tubule using either micropuncture or *in vitro* microperfusion techniques. Early micropuncture studies assessing only bicarbonate concentration in tubule fluid suggested that K^+ depletion increased bicarbonate reabsorption by the proximal convoluted tubule (39,49). A subsequent investigation of rats that had been on a K^+-free diet for 2 weeks and did not exhibit metabolic alkalosis detected an increase in fractional, but not absolute proximal bicarbonate reabsorption (41). Of note, the glomerular filtration rate (GFR) was significantly lower in the K^+-depleted rats, and therefore, a diminished bicarbonate load was presented to the tubule. This issue was circumvented in recent studies by Chan, Biagi, and Giebisch, who employed *in vivo* microperfusion of both the proximal convoluted tubule and the peritubular capillary (12). These investigators found that rats maintained on a K^+-free diet for 4–5 weeks, which had developed sustained metabolic alkalosis, exhibited a 20% increase in HCO_3 reabsorption by the proximal convoluted tubule. Thus, it appears that K^+ depletion of sufficient severity does increase the capacity of the

proximal tubule for HCO_3 reabsorption. However, the increased capacity for HCO_3 reabsorption appears to be inadequate to explain the maintenance of an elevated plasma HCO_3 level of almost twice normal, suggesting that either a decrease in GFR or increased HCO_3 transport at more distal nephron sites is also required to sustain the alkalosis (14).

Furthermore, it is of interest that these investigators found no change in proximal tubule bicarbonate reabsorption in response to acute changes in peritubular K^+ concentration ranging between 2 and 6 mEq/liter (12). A decrease in potassium concentration also does not stimulate HCO_3 reabsorption by proximal tubules perfused *in vitro* (55). However, Levine *et al.* (41) found that an acute intravenous KCl load sufficient to raise plasma K^+ concentration from 3.5 to 4.5 mEq/liter decreased both fractional and absolute HCO_3 reabsorption by the proximal tubule. One possible explanation for these disparate findings is that the response of HCO_3 reabsorption to an intravenous K^+ load is mediated by effects independent of plasma K^+ concentration per se, but additional studies are required to resolve this issue.

To summarize, as indicated in Table II, chronic potassium depletion appears to increase HCO_3 reabsorption by the proximal tubule. The response to ingestion of a high K^+ diet has not been examined directly, but there is no evidence that a chronic high potassium intake alters plasma HCO_3 concentration in normal men or dogs (70). A low K^+ concentration per se does not seem to increase HCO_3 reabsorption by the proximal nephron, but the response to hyperkalemia is unclear. The data suggest that an acute intravenous infusion of KCl reduces HCO_3 reabsorption, but whether the response is mediated directly by the increase in plasma K^+ concentration is unresolved.

IV. MAINTENANCE OF NORMAL H^+ HOMEOSTASIS DURING VARIATIONS IN DIETARY K^+ INTAKE

Given these observations, it is of interest to speculate concerning how K^+-induced changes in acidification mechanisms might function to maintain normal H^+ homeostasis with physiological modifications in K^+ intake and also lead to derangements in H^+ homeostasis with pathological deviations in K^+ balances.

Ingestion of a low K^+ diet decreases aldosterone secretion and may also result in increased sodium reabsorption at proximal nephron sites (4,12,31). These effects, which diminish sodium delivery to the distal nephron and concurrently decrease the ambient mineralocorticoid level, should both decrease K^+ secretion. In addition, however, they would tend to decrease H^+ secretion by the distal nephron. An increase in NH_3 production and excretion should serve as a counterbalance by stimulating H^+ secretion and in so doing might result in yet further K^+ sparing. Finally, a direct increase in the distal nephron H^+ pump

would also tend to sustain H^+ homeostasis. It should be appreciated that this hypothesis simply represents a refinement of the regulatory scheme proposed originally by Dr. Berliner, which suggested that K^+ and H^+ compete for secretion at distal nephron sites (5,6).

With a high K^+ diet, the sequence of events is just the opposite. Aldosterone secretion is stimulated and sodium delivery to the distal nephron is enhanced (8,11,12,41,54,72). Both these effects should increase K^+ secretion distally, but also would tend to stimulate H^+ secretion. This latter effect is counterbalanced by a decrease in NH_3 production. Additional data are needed to delineate whether decreases in proximal HCO_3 reabsorption and in the distal nephron H^+ secretory capacity also occur under physiological settings and further act to counterbalance the stimulatory effects on H^+ secretion.

V. METABOLIC ALKALOSIS SECONDARY TO K^+ DEPLETION

While potassium depletion is an invariable feature of chronic metabolic alkalosis, the precise role of altered potassium homeostasis in inducing and sustaining the alkalosis has been a subject of considerable debate. In part, this has resulted from divergent species response. In the dog, potassium depletion results in metabolic acidosis secondary to a decrease in aldosterone secretion (9,18,26), although it does appear to potentiate the alkalosis-producing effect of excess mineralocorticoids in this species (24). By contrast in the rat, whereas moderate degrees of K^+ depletion do not cause alkalosis (41), the evidence that severe pure K^+ depletion results in metabolic alkalosis is quite convincing (12,13, 18,67). In humans, moderate degrees of K^+ depletion may result in a slight increase in plasma HCO_3 concentration (less than 4 mEq/liter) (30,65). However, the limited data available suggest that severe K^+ depletion can result in significant metabolic alkalosis in humans (17,35).

As summarized in Fig. 6, all of the factors delineated above may play a role in both inducing and sustaining the alkalosis, including increased NH_3 production, an increase in H^+ secretory capacity of the distal nephron, and an increase in HCO_3 reabsorption by the proximal nephron. In addition, the decrease in GFR induced by severe K^+ depletion may play a role in sustaining the alkalosis (14,42).

VI. METABOLIC ACIDOSIS SECONDARY TO HYPERKALEMIA

While a high potassium diet does not result in any abnormalities in acid–base homeostasis under normal conditions (70), hyperkalemia can result in metabolic

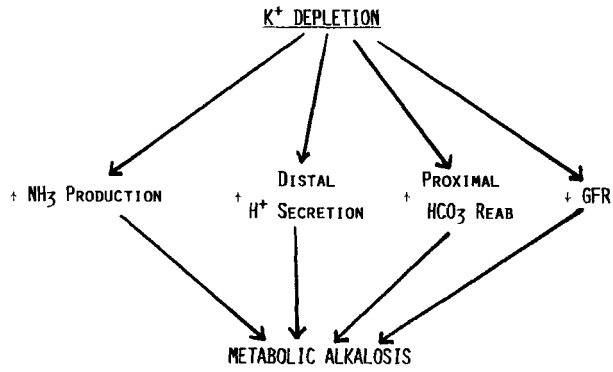

FIG. 6. Mechanisms for the induction and maintenance of metabolic alkalosis with K^+ depletion. See text for discussion.

acidosis in certain abnormal settings (23,27,44,55,61). In humans with hyporeninemic hypoaldosteronism, hyperkalemia in association with hyperchloremic acidosis is a common finding (44,55,61). Normalizing the serum K^+ by either the use of oral cation exchange resins or by reducing dietary K^+ intake returns plasma HCO_3 concentration to normal, suggesting that hyperkalemia plays an important role in sustaining the acidosis. The improvement in acid–base status is accompanied by changes in urine pH and ammonium excretion consistent with an increase in renal ammonia production (44,55,61).

Although correction of hyperkalemia does not improve metabolic acidosis in mineralocorticoid-deficient dogs (25), recent studies have shown that hyperkalemia can provoke acidosis in adrenalectomized dogs if the availability of the urinary buffer phosphate is concurrently restricted (27).

Thus, it seems clear that a high potassium can promote metabolic acidosis under conditions where other H^+ regulatory mechanisms are compromised, as, for example, GFR, aldosterone secretion, or the availability of other urinary buffers. As summarized in Fig. 7, a decrease in ammonia production appears to be the major acidosis-inducing event. Whether concurrent alterations in the H^+ secretory mechanism at either proximal or distal nephron sites also play a role in producing acidosis has not been delineated.

VII. CONCLUSION

To summarize, alterations in potassium can modify urinary acidification by influencing renal NH_3 production, the H^+ secretory mechanism at distal nephron sites, and bicarbonate reabsorption by the proximal tubule. Under normal conditions, this interplay serves to sustain acid–base homeostasis in the face of deviations in potassium intake. However, with pathological degress of K^+

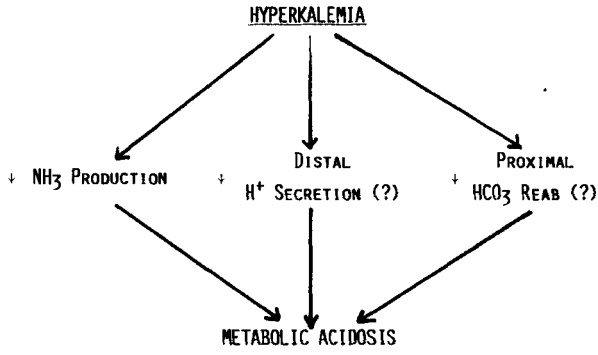

FIG. 7. Mechanisms for the induction and maintenance of metabolic acidosis with hyperkalemia. See text for discussion.

depletion or hyperkalemia, these same mechanisms can result in metabolic alkalosis or acidosis.

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Chapter 11

Renal Potassium Adaptation: Cellular Mechanisms and Morphology

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I. INTRODUCTION

Potassium is one of the most abundant cations in the body and is critical for cell functions. Cell growth, division, volume regulation, and acid–base balance depend on a high intracellular potassium concentration. Furthermore, the high potassium concentration in cells and the low concentration in the extracellular fluid contribute to the electrical properties of cell membranes in both excitable and nonexcitable tissue. Changes in cell potassium modify intracellular pH, and thus potassium can indirectly influence a variety of metabolic processes by altering intracellular pH.

These vital functions depend on a variety of regulatory mechanisms that serve to maintain total body potassium content and the distribution of potassium between intracellular and extracellular fluids. Maintenance of potassium content requires a balance between intake and excretion, so-called external potassium balance. Potassium excretion is accomplished primarily by the kidneys which excrete 90–95% of ingested potassium in a wide variety of conditions. Illustrating the dynamic range of these mechanisms regulating external potassium balance, potassium excretion can increase over 20-fold to equal intake with little or no change in body potassium content. The colon excretes the balance of the ingested potassium, and its contribution to potassium homeostasis is reviewed by Hayslett *et al.* (this volume, Chapter 21). The distribution of potassium between the intracellular and extracellular fluids, so-called internal balance, is regulated by a variety of hormones and by the acid–base status. Some aspects of hormonal regulation of internal balance will be discussed in this chapter in the context of potassium adaptation; however, for a more complete description the reader is referred to DeFronzo (this volume, Chapter 13).

This review will focus on the cellular mechanisms of renal potassium adaptation which is defined as the increased ability to excrete potassium in response to an acute infusion of KCl. Recent data will be reviewed, with special emphasis on information derived from morphological observations, and electrophysiological, transport, and biochemical studies.

II. OVERVIEW OF POTASSIUM ADAPTATION

A. Renal and Extrarenal Adaptation

In 1947, Thatcher and Radike found that after 12 days of chronic oral potassium loading, rats were able to survive acute potassium challenges that were lethal to animals on a normal diet. They called this phenomenon "potassium tolerance" (or adaptation) and speculated that both increased renal potassium excretion and some undefined nonrenal factors may play a role in its development. Furthermore, they observed that chronic deoxycorticosterone acetate (DOCA) administration also produced tolerance.

Three years later, Berliner *et al.* (1950) first demonstrated a renal contribution to potassium adaptation. During acute infusion of KCl, the rate of potassium excretion was substantially increased in dogs given a high potassium diet for 2 weeks compared with controls (Fig. 1). Since the rate of potassium excretion was greater than the amount filtered, these data also confirmed previous observations demonstrating potassium secretion by the renal tubule (Berliner and Kennedy, 1948; Mudge *et al.*, 1948; Berliner, 1961). Several investigators have subsequently demonstrated potassium adaptation in rat (Adam *et al.*, 1968, 1977, 1984; Wright *et al.*, 1971; Schon *et al.*, 1974, 1981; Wright, 1977; Silva *et al.*, 1977; Stanton *et al.*, 1981, 1985a,b; Hayslett and Binder, 1982; Stanton and Giebisch, 1982b; Bengele *et al.*, 1983; Hirsch *et al.*, 1984; Goldstein *et al.*, 1985), mouse (Doucet and Katz, 1980), rabbit (Fine *et al.*, 1979), and in the isolated and perfused kidney from potassium-loaded rats (Silva *et al.*, 1975).

These seminal observations by Berliner and co-workers (1950) not only demonstrated the importance of the kidney in maintaining potassium homeostasis during increased potassium intake, but the data also revealed that an extrarenal mechanism may contribute to the homeostatic response. Plasma potassium levels were reduced in animals ingesting the high-potassium diet, which suggests that extrarenal uptake of potassium was elevated in these animals (Fig. 1). Similar observations were made by Alexander and Levinsky (1968) in rats fed a high potassium diet for 4–6 days. In this study, animals were nephrectomized before an acute dose of potassium: during KCl loading, the increase in plasma potassium levels was reduced in animals fed the high potassium diet. These results clearly demonstrated enhanced extrarenal uptake of potassium in adapted rats. Subsequently, Bia and DeFronzo (1982) showed that extrarenal potassium adap-

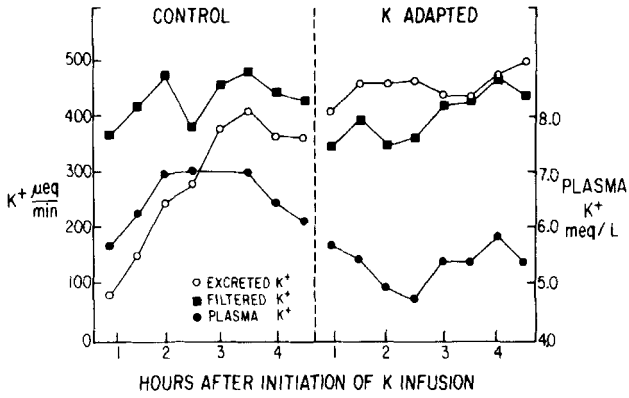


FIG. 1. Effects of a high potassium diet (K adapted; 8 g KCl/day for 2 weeks) on renal and extrarenal handling of potassium in dog. KCl was infused at the rate of 500 μEq/min during clearance experiments. Creatinine clearances were not different in the two groups. [Modified from Berliner *et al.* (1950).]

tation, which involved enhanced uptake of potassium by skeletal muscle and liver cells, was dependent upon an increase in mineralocorticoid hormone levels: adrenalectomy enhanced the development of hyperkalemia, whereas physiological doses of aldosterone restored the change in plasma potassium to control levels. (Bia *et al.*, 1982; Young and Jackson, 1982) (Fig. 2). Thus, potassium

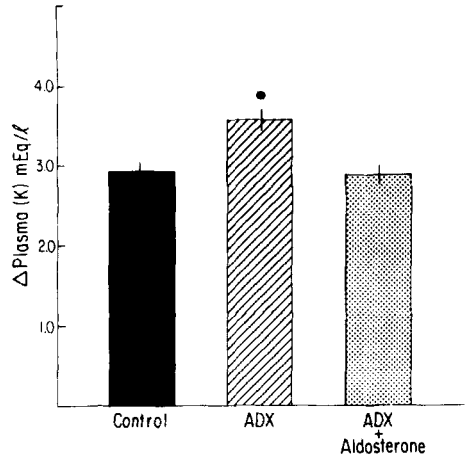


FIG. 2. Extrarenal potassium homeostasis. Adrenalectomy (ADX) was performed 7–10 days before study. Rats received either vehicle or aldosterone (2.5 μg/day) by osmotic minipump for 7–10 days. On the day of study, the rats were bilaterally nephrectomized and 0.17 mEq KCl/100 g body weight was infused intravenously over 1 hr. ΔPlasma (K) is the increase in plasma potassium concentration. $*P < 0.005$ versus control and ADX + aldosterone. [Modified from Bia and DeFronzo (1981).]

adaptation is characterized by enhanced urinary excretion of potassium as well as by accelerated extrarenal uptake of this cation.

In the first 4 hr following potassium infusion, the renal and extrarenal mechanisms contribute about equally to buffering the ensuing hyperkalemia (Bia and DeFronzo, 1981; Smith *et al.*, 1985). Approximately half of the infused potassium is excreted by the kidneys and the remainder is sequestered inside cells; a small fraction remains in the extracellular fluid. Thereafter, the kidney continues to excrete potassium at an accelerated rate until the surfeit is eliminated and potassium balance is restored. It should be emphasized that in many conditions adaptation is not associated with a continuously high level of potassium excretion; the rate of excretion between meals or experimentally induced challenges of potassium is often similar in controls and adapted animals (see Fig. 1; Wright *et al.*, 1971).

B. Biological Significance of Adaptation

Although potassium adaptation has been produced in the examples cited above in experimental settings, renal adaptation may develop and contribute to homeostasis during normal variations in potassium intake as well as during ingestion of salt substitutes which contain KCl, administration of therapeutic doses of potassium penicillin, and during prolonged tissue damage (Smith *et al.*, 1985). Furthermore, administration of drugs or alterations of hormone levels that alter the internal distribution of potassium, such as β-blocking agents, could also induce renal adaptation by producing hyperkalemia. Finally, during chronic renal failure, the remnant-functioning nephrons must increase potassium excretion to maintain potassium balance and, as such, adapt. (See Section III,D for a more complete discussion.) The ability of the kidney to adapt to alterations in either external or internal potassium balance increases the survivability of an organism to subsequent challenges of potassium.

III. RENAL TUBULE SITES AND MECHANISMS OF RENAL POTASSIUM ADAPTATION

A. Potassium Transport by the Nephron

Micropuncture and microperfusion studies *in vitro* and *in vivo* have shown that ~70% of the potassium filtered by the glomerulus is reabsorbed by the proximal tubule, and that an additional 20% is reabsorbed by the loop of Henle (reviewed by Wright, 1977, this volume, Chapter 6; Giebisch, 1978; Stanton and Giebisch, 1981). In most conditions, therefore, ~10% of the filtered potassium reaches the

early distal tubule. It is the distal[1] and collecting tubule that regulate the amount of potassium appearing in the urine. Depending on the metabolic state, potassium is either reabsorbed so that excretion is 1–3% of the filtered load, or is secreted by the distal and collecting tubule such that excretion may exceed 150% of that filtered.

Wright *et al.* (1971) first showed directly by free-flow micropuncture that potassium adaptation stimulates potassium secretion by rat superficial distal tubules. Potassium secretion was stimulated during intravenous infusion of KCl compared with control animals but was similar when the animals received Ringer's solution. This augmentation of potassium secretion was subsequently shown to be independent of alterations in tubule flow rate and sodium concentration during normokalemia and hyperkalemia (Stanton *et al.*, 1981; Stanton and Giebisch, 1982b). In adapted animals, the increment in potassium secretion produced by an elevation in plasma potassium concentration was five times greater than the increase observed in control animals. This observation illustrates the tremendous adaptive response of the potassium secretory mechanism (Fig. 3). Potassium secretion is also augmented in the cortical (Fine *et al.*, 1979) and medullary collecting duct (Schon *et al.*, 1981) in adapted animals.

Whereas the contribution to potassium excretion by the distal nephron during adaptation has been well characterized, considerably less is known concerning the proximal tubule and loop of Henle. Wright *et al.* (1971) found that the fractional delivery of potassium to the early distal tubule was not significantly augmented in animals ingesting a potassium-rich diet during acute KCl infusion compared with controls, which suggests that the proximal tubule and loop of Henle did not contribute to the increase in urinary potassium excretion. Jamison and co-workers (Battilana *et al.*, 1978; Sufit and Jamison, 1983; Jamison and Muller-Suur, this volume, Chapter 7) have suggested that potassium recycling from the medullary collecting duct to the pars recta of the proximal tubule and descending thin limb of Henle's loop is enhanced during potassium adaptation which, in turn, may augment urinary potassium excretion.

According to this theory, as potassium secretion is enhanced along the cortical collecting duct, relatively more potassium is passively reabsorbed into the medullary interstitial fluid which elevates the potassium concentration. As a consequence, the passive entry of potassium into the lumen of the pars recta and thin descending limb, especially of juxtamedullary nephrons, is elevated. Both increased delivery of potassium into the thick ascending limb of Henle's loop and hyperkalemia inhibit net potassium and sodium reabsorption by this nephron

[1] The superficial distal tubule is composed of three structurally distinct segments (see Fig. 6 and Section III,B for a more complete discussion). Microperfusion studies have shown that the majority of potassium secretion is restricted to cells of the late distal tububle which is composed of the connecting and initial collecting tubule segments (Stanton *et al.*, 1981; Stanton and Giebisch, 1982a,b).

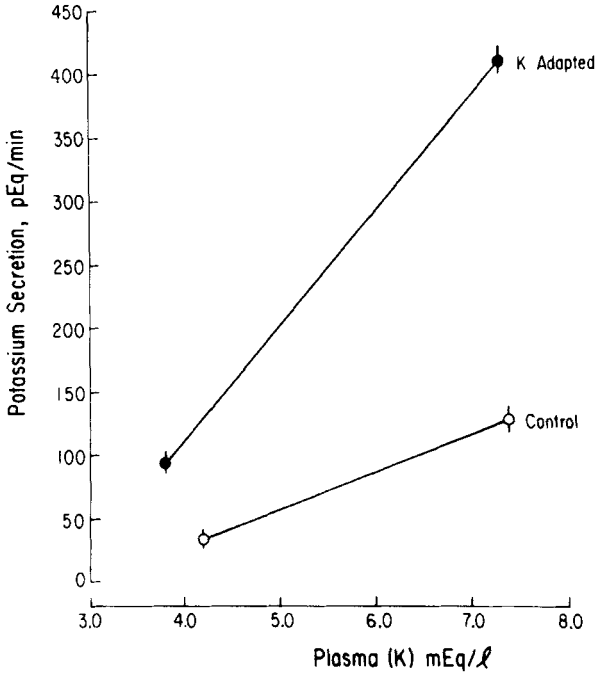


FIG. 3. Effect of K adaptation on potassium secretion by the superficial distal tubule. Secretion was measured by continuous microperfusion in control and K-adapted animals (4 weeks of a K-supplemented diet: 15 g KCl added to each 100 g food, 0.1 *M* KCl to water) during normokalemia and hyperkalemia produced by intravenous infusion of KCl. [Data from Stanton and Giebisch (1982b).]

segment (Stokes, 1982). This would increase the delivery of potassium into the distal tubule during adaptation thereby stimulating urinary potassium excretion. Furthermore, a reduction in sodium reabsorption by the ascending limb of Henle's loop would, by reducing the medullary interstitial osmolarity, decrease passive water reabsorption by the thin descending limb of Henle's loop, thereby augmenting fluid delivery into the distal and collecting tubule. Because potassium secretion is stimulated by enhanced tubule flow rate, this would enhance potassium secretion in these segments. Although Wright *et al.* (1971) did not find evidence for this hypothesis in superficial nephrons of adapted rats, Jamison and Müller-Suur (see Chapter 7, this volume) have suggested that this mechanism would be more likely to occur in juxtamedullary nephrons whose early distal tubules are not accessible to micropuncture. It is likely, however, that recycling only contributes importantly to increased potassium excretion during acute alterations in potassium secretion by the collecting tubule and is relatively less important during steady-state conditions.

Little is known about the effects of chronic potassium loading on the proximal tubule. A direct effect of dietary potassium loading on the proximal convoluted tubule cannot be ruled out since Brandis *et al.* (1972) found that acute hyperkalemia inhibits fluid and thus potassium reabsorption by the proximal tubule of rat. It is not known, however, whether chronic hyperkalemia associated with a high potassium diet leads to any alteration in the transport capacity of the proximal tubule in adapted animals. Since there are no mineralocorticoid receptors in the proximal tubule it is unlikely that the increase in aldosterone during adaptation influences the function of this nephron segment (Farman *et al.*, 1981; Doucet and Katz, 1980).

B. Regulation of Potassium Excretion

1. Cell Model

To understand how potassium secretion may be enhanced by the distal and collecting duct epithelium during potassium adaptation, the present understanding of the cellular mechanisms of potassium transport will first be reviewed. With the recent application of intracellular microelectrode techniques to the study of single cells of the rabbit collecting duct isolated and perfused *in vitro* (Koeppen *et al.*, 1983; O'Neil and Sansom, 1984a,b; Sansom and O'Neil, 1985) combined with basic transport and electrophysiological techniques, it has been possible to define some of the electrochemical driving forces and membrane conductances for transcellular and paracellular ion movement by the initial and cortical collecting duct and to construct a cell model to account for transepithelial potassium and sodium movement (Fig. 4; Giebisch, this volume, Chapter 8).

The initial collecting (i.e., late distal tubule) and cortical collecting tubule reabsorb sodium from and secrete potassium into the tubule lumen (Grantham *et al.*, 1970; O'Neil and Helman, 1977; Schwartz and Burg, 1978; Stoner *et al.*, 1974; Kunau *et al.*, 1974, 1978; Good and Wright, 1979; Stanton *et al.*, 1981; Stanton and Giebisch, 1982a; Velazquez *et al.*, 1984; Ellison *et al.*, 1985). Inasmuch as both ions are distributed across the epithelium against their electrochemical gradients, they are actively transported.

The transepithelial voltage of the collecting duct is oriented lumen negative under most conditions and depends on the active reabsorption of sodium (Grantham *et al.*, 1970; Koeppen and Helman, 1982; O'Neil and Helman, 1977; Stoner *et al.*, 1974; Stokes, 1981). Sodium transport and the negative transepithelial voltage are inhibited by the addition of amiloride to the luminal perfusion fluid or by the addition of ouabain to the basolateral solution (Grantham *et al.*, 1970; O'Neil and Boulpaep, 1979, 1982; Koeppen and Helman, 1982; Koeppen *et al.*, 1983; O'Neil and Sansom 1984a,b).

The active secretion of potassium also is dependent upon the reabsorption of

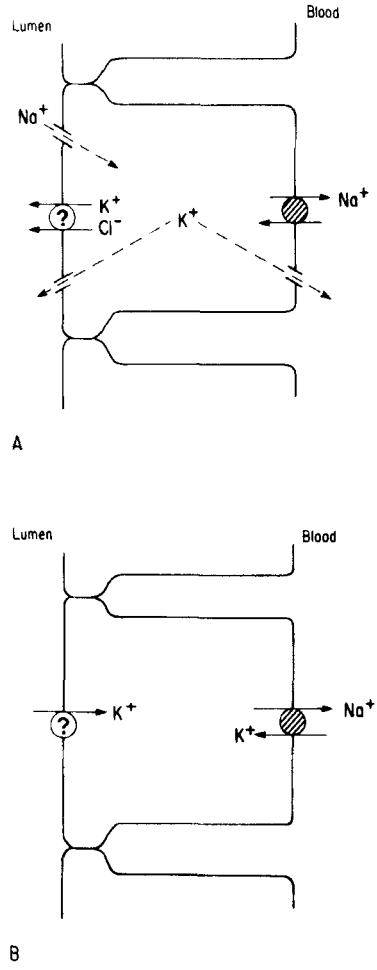


FIG. 4. Cell models of sodium and potassium transport by the collecting tubule. (A) Principal cell. Several lines of evidence suggest that this cell type secretes potassium (see text for details). (B) Intercalated cell. This cell type may absorb potassium, perhaps in exchange for H^+ by an ATP-dependent process (see text for details). Although Na^+,K^+-ATPase is depicted in the basolateral membrane of both cell types, the activity in intercalated cells is low relative to principal cells as demonstrated by Kashgarian *et al.* (1985). [Adapted from Stanton and Giebisch (1981).]

sodium, and inhibition of sodium reabsorption reduces potassium secretion *pari passu* (Grantham *et al.*, 1970; Stoner *et al.*, 1974; O'Neil and Helman, 1977; Stokes, 1981).

Potassium secretion and sodium reabsorption are driven by the Na^+,K^+-ATPase located in the basolateral cell membrane (Fig. 4A). Sodium enters and

potassium exits the cell passively across the luminal membrane down their respective electrochemical gradients through separate conductive pathways. Potassium may also exit the cell across the basolateral membrane through a conductive pathway, thereby allowing a portion of the potassium transported into the cell by the Na^+,K^+-ATPase to be recycled back into the basolateral solution (Koeppen *et al.*, 1983; O'Neil and Sansom, 1984a,b; Sansom and O'Neil, 1985). These transport mechanisms are likely to reside in principal cells, which are the majority cell type in the collecting tubule (Fig. 4A). This conclusion is based on three observations. First, O'Neil and Hayhurst (1985a) have shown, using electrophysiological and optical techniques, that the luminal cell membrane of principal cells has significant conductive pathways to sodium and potassium, whereas the luminal membrane of intercalated cells is poorly conductive to these cations. Furthermore, they showed that these conductances are inhibited by amiloride (Na^+ conductance) and barium (K^+ conductance) agents that also block transepithelial transport of these ions. Second, Kashgarian and co-workers (1985), using monoclonal antibodies to the α-subunit of Na^+,K^+-ATPase, have demonstrated that this enzyme is abundant in principal cells, whereas the amount in intercalated cells is low. Third, ultrastructural studies reveal that chronic mineralocorticoid hormone stimulation of potassium secretion and sodium reabsorption by the collecting duct is accompanied by a specific proliferation of the basolateral membrane of principal cells (Wade *et al.*, 1979; Stanton *et al.*, 1981, 1985a–c; Kaissling, 1982, 1985; Kaissling and LeHir, 1982).

Potassium may also be secreted by an electroneutral KCl cotransport mechanism located in the luminal membrane of the distal tubule (Velazquez *et al.*, 1982; Ellison *et al.*, 1985). This conclusion is based on the observation that reduction of chloride in the luminal fluid stimulates a barium and voltage-insensitive component of potassium secretion (Fig. 4A). It is presently unknown what cell type contains this transport pathway; however, due to the relative abundance of Na^+,K^+-ATPase in principal cells compared with intercalated cells (Kashgarian *et al.*, 1985), it is likely that this electroneutral transport process is located in the former cell type (Fig. 4A).

According to the model summarized in Fig. 4, potassium secretion is coupled to sodium reabsorption by Na^+,K^+-ATPase and is dependent upon the electrochemical gradients for potassium across the cell membranes. Transepithelial secretion of potassium and sodium could be regulated at three key sites. First, cellular uptake of potassium and extrusion of sodium could be altered by a change in the activity of Na^+,K^+-ATPase. This could occur by a change in the total number of enzyme units or by an alteration in the activity of each enzyme. Second, the conductances of the cell membranes to sodium and potassium could be modified. Third, the electrochemical gradients for the passive movement of both ions across the luminal or basolateral membranes could change.

Potassium is also reabsorbed by the collecting duct (Backman and Hayslett,

1983). On the basis of morphological studies demonstrating proliferation of the luminal membrane of intercalated cells during K depletion, a condition accompanied by increased potassium reabsorption in the medullary collecting duct. (Oliver *et al.*, 1957; Toback *et al.*, 1976; Stetson *et al.*, 1980; Evan *et al.*, 1980), it has been suggested that intercalated cells absorb potassium from the tubule fluid into the blood (Stetson *et al.*, 1980; Stanton and Giebisch, 1981). This mechanism, which is not supported by any direct evidence, is depicted in Fig. 4B. Potassium uptake across the luminal membrane must be active, considering the electrochemical gradient across this membrane. It is unlikely that absorption is a secondary active mechanism, e.g., coupled with hydrogen ions because the chemical gradients oppose this mechanism. Thus, potassium reabsorption, if transcellular, occurs via primary active transport or by some other undefined mechanism. Preliminary microelectrode studies of a minority cell type in the collecting tubule, which may represent intercalated cells, reveal that the luminal membrane has no apparent ionic conductances and that the basolateral membrane is primarily conductive to chloride, with no apparent conductive pathway to potassium (Koeppen, 1985). Thus, potassium movement across the basolateral membrane is likely to be via an electroneutral mechanism. It must also be considered that potassium reabsorption in the outer medullary collecting duct may occur via the potassium-selective paracellular pathway driven by the lumen-positive transepithelial voltage (Koeppen, 1985, 1986; Wingo, 1986). Since potassium permeability is enhanced by potassium depletion increased reabsorption of potassium by the paracellular pathway may occur.

2. Adrenal Corticosteroids and Potassium Ions

a. Regulation of Na^+,K^+-ATPase and Basolateral Membrane Area in the Collecting Duct: Cellular Functional Heterogeneity. Several lines of evidence reveal that adrenal corticosteroids play an integral role in the development of K adaptation. As illustrated in Fig. 5, Boyd and Mulrow (1972; Boyd *et al.*, 1971) observed that a relatively small increase in plasma potassium concentration, produced by dietary potassium loading, resulted in a significant increase in aldosterone secretion and, hence, in plasma aldosterone levels. Hyperkalemia is usually only observed immediately following potassium-supplemented meals; several hours after eating plasma potassium is often less in adapted animals (Wright *et al.*, 1971; Stanton *et al.*, 1981, 1982a,b). Nevertheless, even during relative hypokalemia, plasma aldosterone levels are augmented in K-adapted animals (Alexander and Levinsky, 1968; Stanton *et al.*, 1981, 1982a,b; Hayslett and Binder, 1982; Hirsch *et al.*, 1984).

When mineralocorticoid levels are elevated for several days in animals subjected to a high potassium diet, renal potassium excretion is enhanced due to stimulated potassium secretion, primarily by the distal (Kunau *et al.*, 1974;

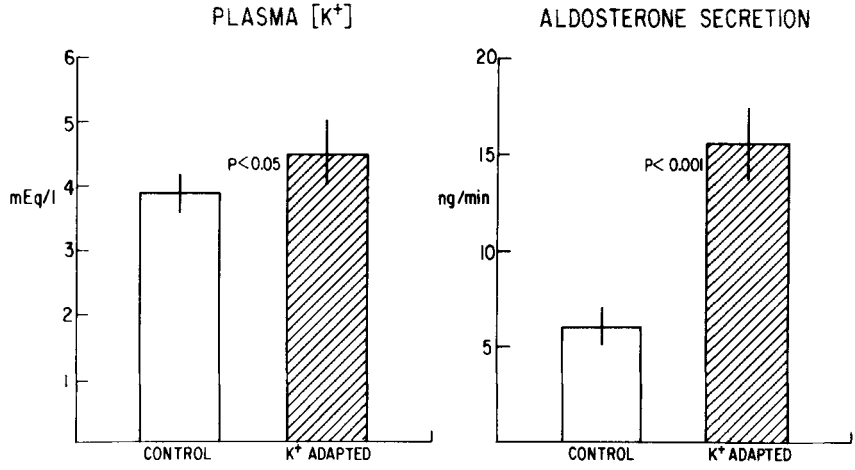


FIG. 5. Relationship between plasma potassium concentration and aldosterone secretion in K-adapted rats. K adaptation was produced by adding 0.3 *M* KCl to the drinking water. [Data from Boyd *et al.* (1971, 1972).]

Peterson and Wright, 1977; Wright *et al.*, 1971; Stanton *et al.*, 1981, 1982a,b) and cortical collecting tubule (O'Neil and Helman, 1977; Schwartz and Burg, 1978; Wade *et al.*, 1978; Fine *et al.*, 1979). This accelerated secretion is attributable, in part at least, to an increase in the activity of Na^+,K^+-ATPase (Doucet and Katz, 1980; El Mernissi *et al.*, 1983a; Garg *et al.*, 1981; Hayslett and Binder, 1982; O'Neil and Hayhurst, 1985b; Mujais *et al.*, 1984a–c, 1985).

Silva and colleagues (1973) and Schon *et al.* (1974) found that chronic potassium loading markedly increased Na^+,K^+-ATPase activity in renal tissue homogenates in both the cortex and medulla, but not in brain, liver, or diaphragm muscle. This tissue-specific effect was subsequently localized to the cortical and medullary collecting duct by Doucet and Katz (1980), who measured the activity of Na^+,K^+-ATPase in single tubules from K-adapted mice.

Initial insight into the effects of mineralocorticoids on the relationship between cell structure and function in the collecting tubule was provided by Wade and coworkers in 1979. Administration of DOCA (5 mg/day) to rabbits for 2 weeks enhanced the basolateral membrane area of principal cells and stimulated both sodium reabsorption and potassium secretion. This change in cell structure was specific to the basolateral membrane of principal cells and correlated with a specific increase in Na^+,K^+-ATPase activity in the collecting tubule (Garg *et al.*, 1981; El Mernissi *et al.*, 1983a). Intercalated cell structure and luminal membrane of principal cells were not affected by DOCA. So specific is this response that several other enzymes located in the basolateral membrane were not stimulated by mineralocorticoid treatment (El Mernissi *et al.*, 1983a).

Potassium adaptation and the associated increase in aldosterone are also associated with profound and specific alterations in the ultrastructure of cells in the distal tubule. Figure 6 is a schematic representation of the distribution of cell types in the distal tubule which is the portion of the nephron between the macula densa and the confluence with another tubule. This nephron segment is heterogeneous composed of three segments: distal convoluted, connecting, and initial collecting tubule, and four cells types (Crayen and Thoenes, 1978; Woodhall and Tisher, 1973; Bengele *et al.*, 1978; Kaissling and Kriz, 1979; Stanton *et al.*, 1981). Microperfusion studies of discrete segments in the superficial distal tubule of rat have shown that most if not all potassium secretion occurs in the connecting and initial collecting tubule and that potassium secretion and sodium reabsorption are enhanced by K adaptation (Stanton *et al.*, 1981; Stanton and Giebisch, 1982b). Both the initial and cortical collecting tubule are composed of principal and intercalated cells and these tubule segments share many functional characteristics, including mineralocorticoid receptors and aldosterone stimulated increases in sodium reabsorption and potassium secretion (Gross *et al.*, 1975, 1977; Koeppen and Giebisch, 1985; O'Neil and Helman, 1977; Farman *et al.*, 1981; Field *et al.*, 1984, 1985; Garg *et al.*, 1981, 1985; Sansom and O'Neil, 1985; Kaissling and Lehir, 1982; Allen and Barratt, 1981; Stokes *et al.*, 1981; Stanton, 1985c; Stanton *et al.*, 1981, 1982b, 1985a,b).

Chronic elevation of plasma aldosterone, produced by several weeks of a high potassium diet, augments the area of the basolateral membrane in connecting tubule and principal cells in the superficial distal tubule of rat kidney (Fig. 7;

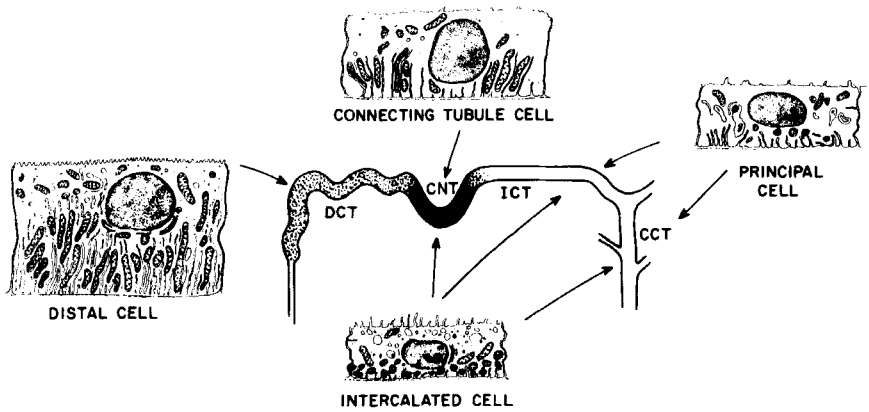


FIG. 6. Schematic of the distribution of cell types within the superficial distal tubule of the rat kidney. The transitions between the distal convoluted tubule (DCT) and connecting tubule (CNT) segments and between the latter segment and the initial collecting tubule (ICT) segment are gradual in rat and man, but are more abrupt in rabbit. [From Stanton (1985c).]

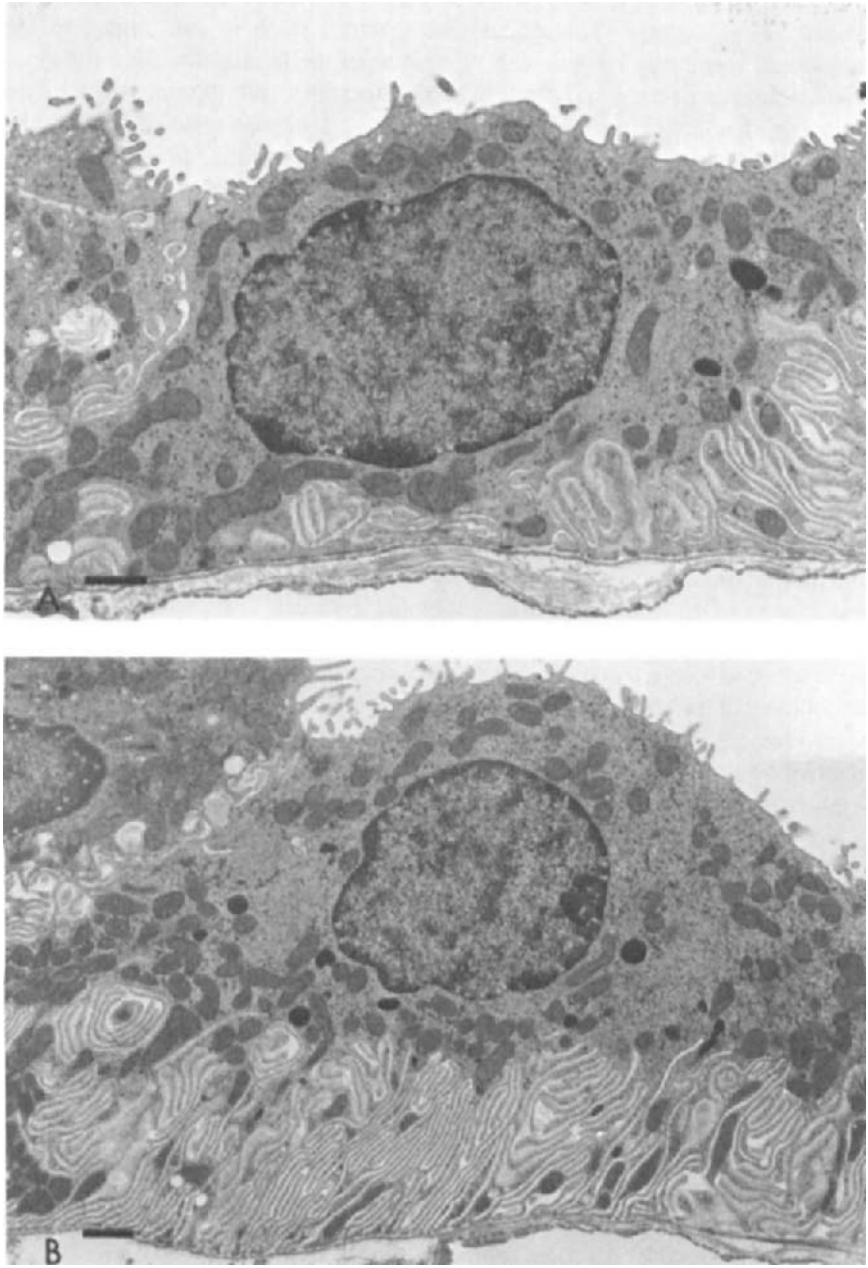


FIG. 7. Electron micrographs of principal cells from initial collecting tubule of control (A) and (B) K-adapted rat. The length of the basolateral membrane increased from 162 to 455 μm ($P < 0.01$) after 4 weeks of ingesting a K-rich diet (bar = 1 μm). [Data from Stanton *et al.* (1981).]

Stanton *et al.*, 1981). This effect was specific, since it was limited to the basolateral membrane of these cell types; neither the luminal nor the basolateral membrane area of the distal convoluted or intercalated cell type was affected by potassium loading (Fig. 7). Similar results have also been observed in the cortical collecting tubule of rabbits ingesting a high potassium, low sodium diet. Since the response in the latter study was inhibited by canrenote K, an inhibitor of mineralocorticoid action and was reproduced by chronic DOCA treatment, Kaissling and Lehir (1982) concluded that the ultrastructure of principal cells was regulated by mineralocorticoid hormones. In addition, Lehir *et al.* (1982) have demonstrated that these changes in membrane area correlate with an increase in the activity of Na^+,K^+-ATPase. Principal cells in the medullary collecting duct of rat also respond to K adaptation by increasing the area of the basolateral membrane (Rastegar *et al.*, 1980). Figure 8 is a summary of the effects of a high potassium diet on the basolateral membrane area of distal nephron cells.

Taken together, the observations cited above suggest that potassium is secreted and sodium is absorbed by connecting tubule and principal cells and that the proliferation of the basolateral membrane occurs as a result of an increase in the amount of Na^+,K^+-ATPase. Additional evidence to support these conclusions comes from two observations. First, O'Neil and Hayhurst (1985a) have demonstrated by electrophysiological and optical techniques that the luminal cell membrane of principal cells in the cortical collecting tubule of rabbit have significant conductive pathways to potassium and sodium ions. The luminal membrane of intercalated cells did not contain conductive pathways to these ions. Second, in an elegant study, Kashgarian *et al.* (1985) have shown that most

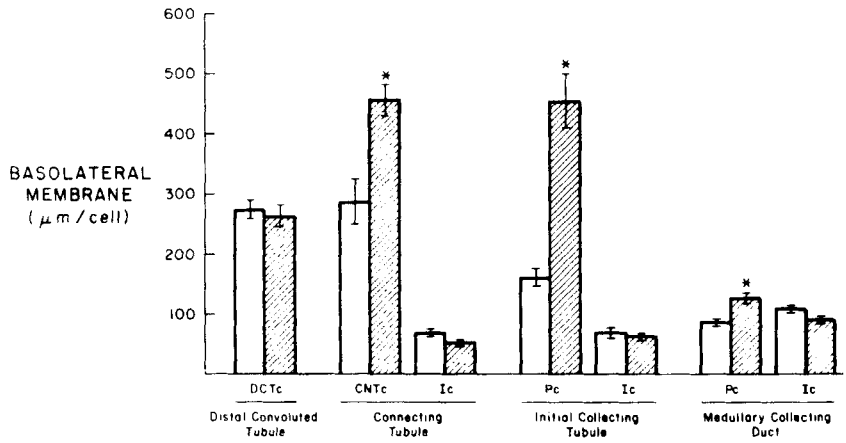


FIG. 8. Effects of K adaptation on the length of the basolateral membrane in the distal nephron. DCTc, distal convoluted tubule cell; CNTc, connecting tubule cell; Ic, intercalated cell; Pc, principal cell. [Data from Stanton *et al.* (1981); Rastegar *et al.* (1980).]

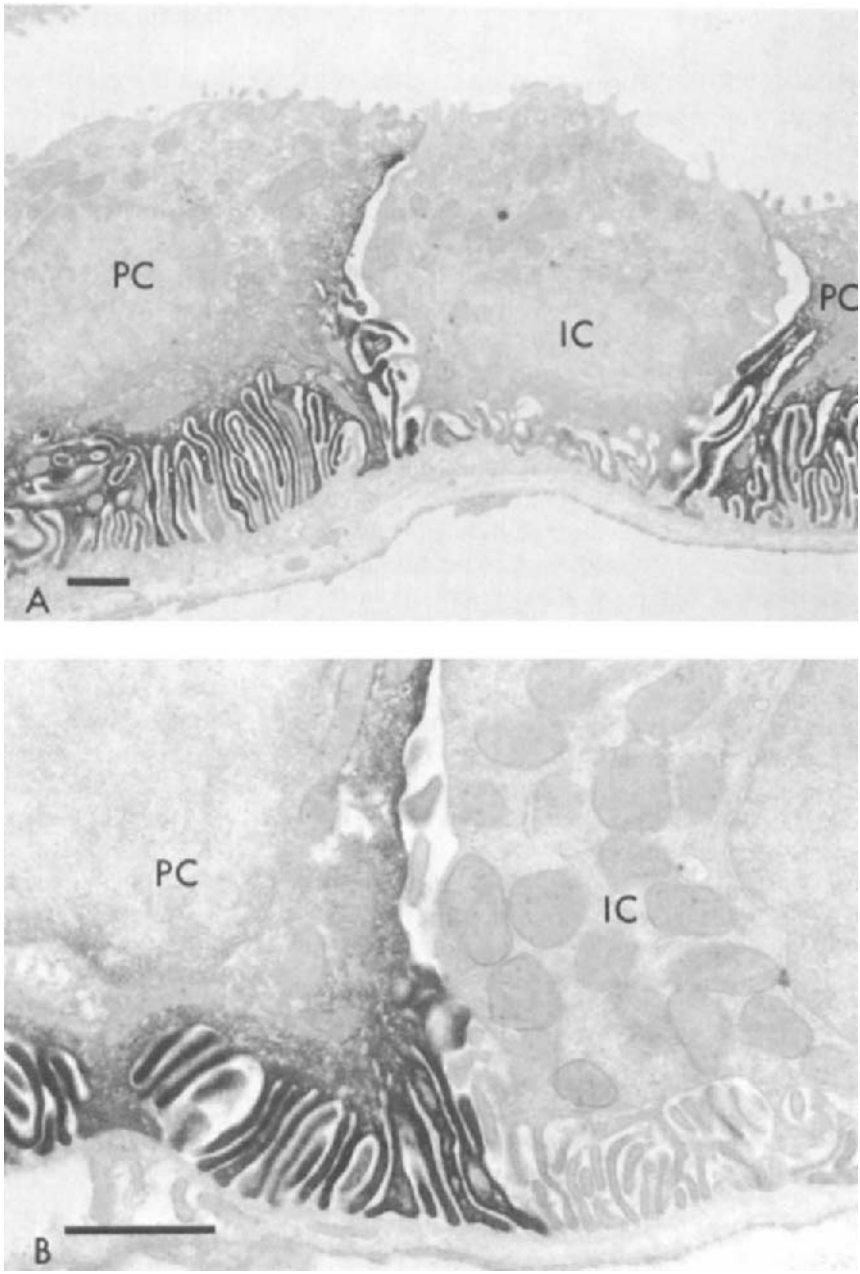


FIG. 9. Distribution of Na^+,K^+-ATPase in cortical collecting tubule of rat (tissue fixation with periodate, lysine, paraformaldehyde) demonstrated by labeling (peroxidase) a monoclonal antibody that binds to the α subunit of the enzyme. (A) Electron micrograph depicting an intercalated cell (IC) between two principal cells (PC). The electron-dense reaction product over the basolateral membrane of principal cells illustrates the distribution of Na^+,K^+-ATPase. Bar = 1 μm. (B) High-power micrograph of collecting tubule. Bar = 1 μm.

of the Na^+,K^+-ATPase in the connecting and collecting tubule is present in the basolateral membrane of the connecting tubule and principal cells. This is demonstrated in Fig. 9 in which the distribution of Na^+,K^+-ATPase was manifest by exposing the tissue to a monoclonal antibody that binds to the α subunit of the enzyme. The monoclonal antibody, labeled with a peroxidase-conjugated secondary antibody, appears as an electron-dense reaction product over the α subunit of the Na^+,K^+-ATPase. Thus, taken together these data suggest that potassium is secreted and sodium absorbed by principal and connecting tubule cells and that the increase in transport following elevations in mineralocorticoid levels is due, in part at least, to an increase in the activity of Na^+,K^+-ATPase.

These studies have not only greatly increased our understanding of the regulation of potassium transport by the collecting tubule, but they also raise two questions. First, since supraphysiological doses of hormone were used in many studies, will changes in membrane area, ion transport and Na^+,K^+-ATPase activity also occur with more physiological stimuli and is the response mediated through mineralocorticoid or glucocorticoid receptors? Second, what role does potassium per se have in the development of the adaptive response to a high potassium diet?

Studies in our laboratory on rats receiving a normal potassium diet have examined the effects of adrenalectomy and selective, physiological hormone replacement (10 days) on the ultrastructure of principal cells in the initial collecting tubule and on the ability of the kidney to excrete an acute intravenous infusion of KCl. The results are depicted in Figs. 10 and 11. Adrenalectomy, with no hormone replacement, sharply reduced both the area of the basolateral membrane and potassium excretion. Whereas dexamethasone[2] replacement in adrenalectomized animals had no effect on membrane area, potassium excretion increased a small but significant amount. When urine flow rate was held constant by experimental design (Stanton *et al.*, 1985b) or when tubule flow rate was controlled by microperfusion of single tubules (Schwartz and Burg, 1978; Field *et al.*, 1984), dexamethasone had no effect on urinary potassium excretion or on potassium secretion by either the distal or collecting tubule. Thus, although glucocorticoids often lead to a kaliuresis (Bartler and Fourman, 1962; Field *et al.*, 1984; Bia *et al.*, 1982; Higashihara and Kokko, 1985), the kaliuretic effect can be ascribed to their effect on urine flow rather than to a direct effect at the level of the distal or collecting tubule. This is underscored by the inability of dexamethasone to influence the structure of principal cells (Figs. 10 and 11) and

[2] Although dexamethasone is not an endogenous steroid, it was used due to its relative high affinity for the glucocorticoid receptor versus the endogenous glucocorticoid in rat, corticosterone. Thus, at low doses any effect could be attributed to interaction with the glucocorticoid receptor. The dose used, 1.2 μg/100 g body weight/day, was the lowest that maintained plasma insulin and glucose concentration and glomerular filtration rate at levels measured in normal adrenal-intact animals (Stanton *et al.*, 1985b).

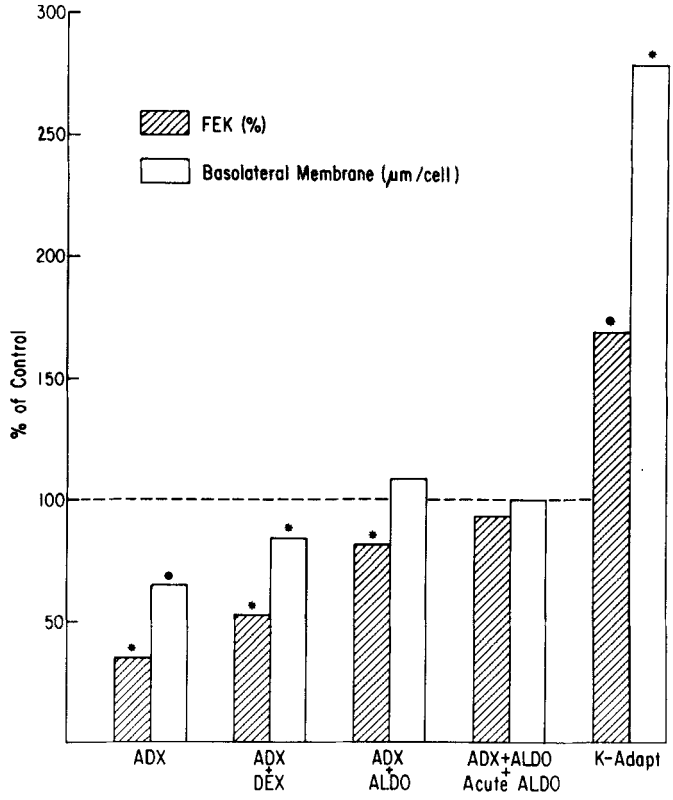
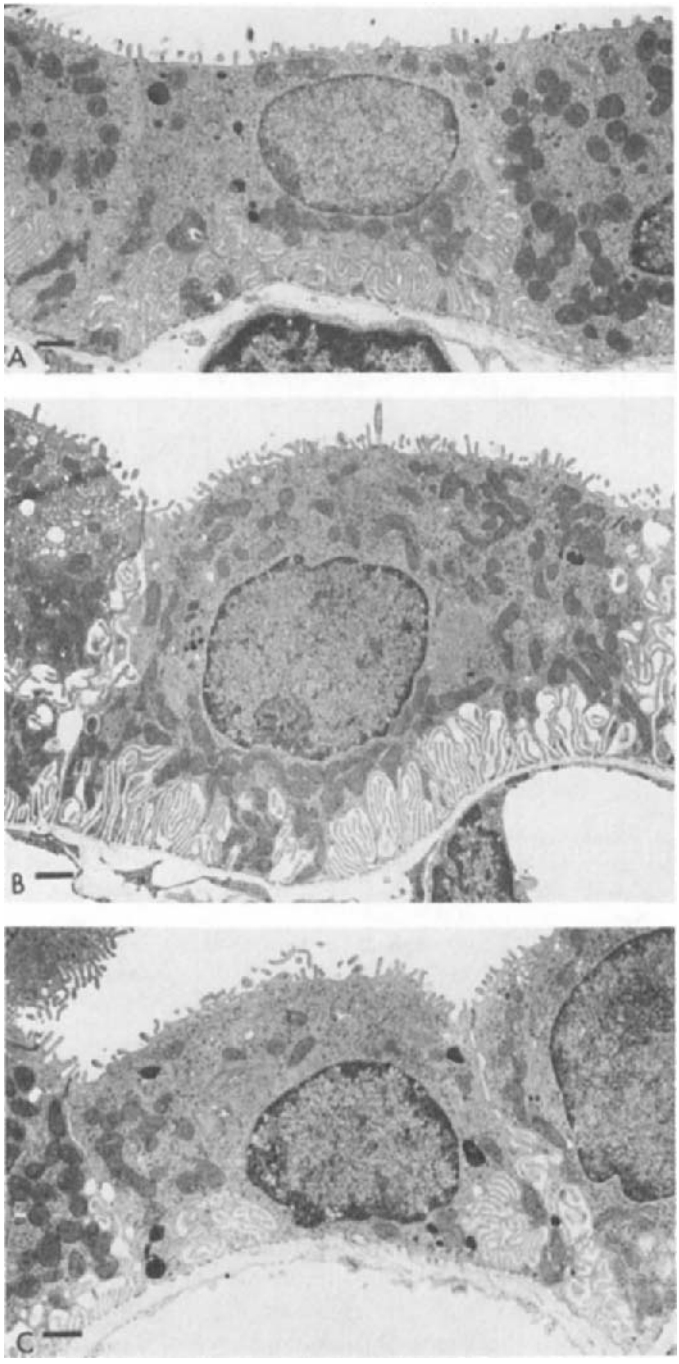


FIG. 10. Relationship between the length of the basolateral membrane of principal cells in the initial collecting tubule and the fractional excretion of potassium as a function of adrenal corticosteroid levels. Both potassium excretion and membrane length are plotted as a percentage of control. ADX, Adrenalectomy (animals studied 10 days after surgery); DEX, dexamethasone replacement in ADX (1.2 μg/100 g body weight/day for 10 days); ALDO, aldosterone replacement in ADX (0.5 μg/100 g body weight/day for 10 days); acute ALDO (0.2 μg/100 g body weight/hr, 2 hr before study); K-Adapt, adrenal-intact, dietary potassium loading (30 mEq K/day for 10 days). [Data from Stanton *et al.* (1981, 1985a,b).]

Na^+,K^+-ATPase activity in the collecting tubule (Mujais *et al.*, 1984b; El Mernissi and Doucet, 1983; Rayson and Lowther, 1984).

We also found that chronic aldosterone replacement, which restored plasma levels to basal levels, sharply increased potassium excretion and augmented the area of the basolateral membrane of principal cells (Figs. 10 and 11). Although membrane area was restored to control levels the ability to excrete an acute infusion of KCl was slightly but significantly less than control. An acute 15-fold increase in aldosterone levels increased potassium excretion to control levels, how-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C

ever, this acute effect of the hormone was not mediated by a change in basolateral membrane area of principal cells (Stanton *et al.*, 1985c). Thus both chronic and acute changes in aldosterone regulate potassium transport by the initial collecting tubule, although the cellular mechanisms, to be discussed in Section IV, are apparently different. Inasmuch as low doses of aldosterone were used in these studies, it can be concluded that the acute and chronic effects of aldosterone were mediated via binding to the mineralocorticoid receptor and not to the glucocorticoid receptor.[3]

It is of particular interest that these aldosterone-induced alterations in membrane area parallel changes in the activity of Na^+,K^+-ATPase in the collecting tubule (Mujais *et al.*, 1984a,b, 1985). The proliferation of basolateral membrane of principal cells occurring in concert with an increase in the number of Na^+,K^+-ATPase units appears to be an important mechanism whereby cells enhance the transport rates of sodium and potassium. This relationship is also observed in other epithelial cells, including the teleost gill, avian salt gland, and colon (Hayslett *et al.*, this volume, Chapter 21), and appears to be a common mechanism whereby epithelial cells increase the capacity to transport electrolytes (reviewed by Wade *et al.*, 1979; Stanton, 1985).

Despite the importance of aldosterone in the development of adaptation to a high potassium diet, potassium also contributes to the renal adaptive response. Silva *et al.* (1973) showed that a high potassium diet in adrenalectomized rats on a fixed replacement of aldosterone (50 μg/day) enhanced Na^+,K^+-ATPase activity in renal homogenates and concluded that a high potassium diet without changes in aldosterone could induce adaptation. In support of this conclusion Addisonian patients receiving replacement doses of mineralocorticoids (Miller *et al.*, 1975) and adrenalectomized, hormone-deficient rabbits are also capable of tolerating a high potassium diet (Wingo *et al.*, 1982).

[3] This conclusion is permitted based on calculations of receptor occupancy. The percentage occupancy of each class of corticosteroid receptors can be estimated from the plasma concentration of aldosterone and the dissociation constant for receptor-hormone binding. In our study, plasma aldosterone with the low dose of hormone was 4.4 ng/dl. Approximately 20% of the mineralocorticoid receptors were bound to aldosterone whereas less than 1% of the glucocorticoid receptors were bound to aldosterone. Since this latter number is extremely low, the effect must be mediated through binding to mineralocorticoid receptors. At the high dose of aldosterone, 59% of mineralocorticoid receptors are occupied with hormone whereas only 2.7% of the glucocorticoid receptors are hormone-bound.

FIG. 11. Electron micrographs of principal cells from the initial collecting tubule; effects of chronic alterations (10 days) in aldosterone levels within the physiological range (groups given with plasma aldosterone levels). (A) Control, 4.4 ng/dl; (B) adrenalectomy with high-dose aldosterone replacement, 23.5 ng/dl; (C) adrenalectomy, 0 ng/dl, dexamethasone replacement (1.2 μg/100 g/day). Note the increase in the number of ribosomes in (C) compared with (A) and (B). Bar = 1 μm. [From Stanton *et al.* (1985a).]

Studies in our laboratory have examined the independent effects of a high potassium diet and aldosterone on the development of renal potassium adaptation (Stanton *et al.*, 1986). The effects of an independent increase in potassium will be considered first. Adrenalectomized rats were given replacement doses of aldosterone and dexamethasone by osmotic minipump and were placed either on a control diet or on a high potassium diet for 10 days. Compared with animals on the control diet, the high potassium group excreted more potassium during an acute KCl infusion (Fig. 12). The high potassium diet also increased the area of the basolateral membrane of principal cells in the initial collecting tubule. Consistent with these observations, a potassium-rich diet has been shown to stimulate potassium secretion and sodium reabsorption by isolated rabbit cortical collecting tubules (Wingo *et al.*, 1982; Muto *et al.*, 1985) by enhancing the sodium and potassium conductance of the luminal membrane (Muto *et al.*, 1985) and the activity of Na^+,K^+-ATPase (Garg and Narang, 1985). It should be noted, however, that in our studies a high potassium intake did not produce the same degree of adaptation observed in adrenal-intact animals given the same potassium intake.

As discussed below, an increase in aldosterone is also required to produce maximal potassium adaptation. Potassium loading may induce renal adaptation by several mechanisms that are not mutually exclusive. First, potassium could by itself increase the activity of Na^+,K^+-ATPase. Second, potassium loading al-

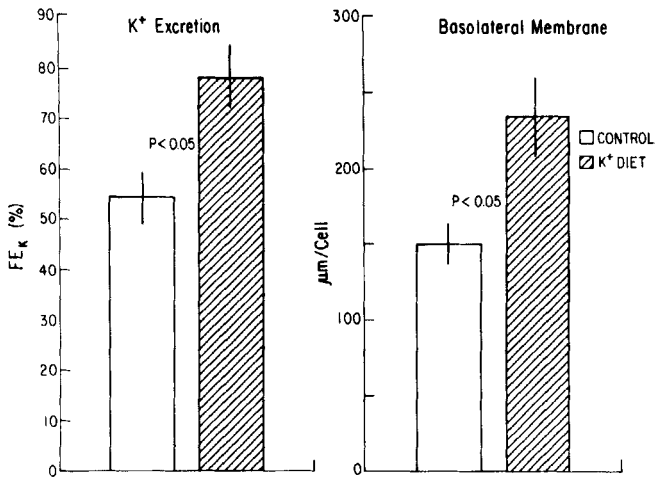

FIG. 12. Effect of a high K diet (20 mEq/day for 10 days) on renal potassium excretion and ultrastructure of principal cells in adrenalectomized aldosterone-replete rats. Both groups of animals were adrenalectomized 10 days before study and received aldosterone (0.5 μg/100 g body weight/day) and dexamethasone (1.2 μg/100 g body weight/day) until the day of experiment. During clearance experiments a KCl solution was infused intravenously. [From Stanton *et al.* (1986).]

ters the metabolism of aldosterone and increases the percentage of biologically active derivatives (Morris, 1985). Third, a high potassium diet increases renal sensitivity to aldosterone (Adam and Funder, 1977), an effect that may be mediated by an increase in the numebr of aldosterone receptors (Rafestin-Oblin *et al.*, 1984).

In the same study, referred to above, we also examined the effects of an independent increase in aldosterone on the development of adaptation. Elevated aldosterone, at normal potassium intake, did not produce adaptation. This observation confirms the original study on potassium adaptation by Berliner and coworkers (1950), but is in contrast to the recent observations of Martin and Hayslett (1986). When we increased both aldosterone and dietary potassium intake, however, potassium excretion was equal to adrenal-intact animals ingesting the same potassium-rich diet. Thus, an increase in both factors is required for the development of the maximal adaptive response (Stanton *et al.*, 1986). It should also be noted that the adrenalectomized animals, with basal aldosterone replacement, tolerated considerably less potassium in the diet than adrenal-intact or adrenalectomized rats receiving the high dose of aldosterone (Stanton *et al.*, 1986).

b. Regulation of Membrane Conductances, Na^+,K^+-ATPase Activity, and Electrochemical Gradients. With the application of intracellular microelectrode techniques to the isolated and perfused cortical collecting tubule of rabbit kidney, considerable progress has been made in elucidating the cellular mechanisms of mineralocorticoid stimulation of transcellular sodium and potassium transport. These studies, to be reviewed below, demonstrate that mineralocorticoids stimulate electrolyte transport by regulating membrane conductances, Na^+,K^+-ATPase activity, and electrochemical driving forces for both sodium and potassium across the luminal and basolateral membrane.

Two weeks of DOCA treatment (5 mg/day) increases net sodium reabsorption and potassium secretion by the collecting tubule, an effect that is accompanied by an increase in the transepithelial equivalent conductances for these ions (O'Neil and Helman, 1977; Koeppen *et al.*, 1983; Koeppen and Giebisch, 1985; Sansom and O'Neil, 1985). DOCA increases the conductance of the luminal membrane to both sodium and potassium by almost twofold. Sansom and O'Neil (1985) have also calculated, by equivalent circuit analysis, that the current carried across the luminal membrane by sodium and potassium increases by about threefold. The sodium influx across this membrane increases due to an elevation of membrane conductance, whereas the potassium efflux increases due to augmentation of both the membrane conductance and the electrical driving force for potassium across this membrane.

Biochemical, morphological, and electrophysiological evidence indicate that the amount of Na^+,K^+-ATPase units increases with chronic hormone stimulation, thereby augmenting the rate of active sodium and potassium transport

across the basolateral membrane (Wade *et al.*, 1979; Garg *et al.*, 1981; Koeppen *et al.*, 1983; O'Neil and Hayhurst, 1985b; Sansom and O'Neil, 1985). Furthermore, since Na^+,K^+-ATPase is electrogenic (Koeppen *et al.*, 1983; Koeppen and Giebisch, 1985; Sansom and O'Neil, 1985), increased activity of this enzyme could account for the observed hyperpolarization of the basolateral membrane voltage. This hyperpolarization alters the passive driving force for potassium movement across the membrane so that potassium may actually enter the cell from the blood inasmuch as the membrane voltage may be greater than the equilibrium potential for potassium. As a result, potassium recycling across the basolateral membrane would decrease further augmenting net potassium secretion. Since the uptake of potassium into the cell across the basolateral membrane is increased intracellular potassium activity may also rise, as shown by Oberleithner and Giebisch (1981), which would also enhance the favorable gradient for potassium secretion through a conductive and possibly an electroneutral pathway in the luminal cell membrane (see Fig. 4A).

Sansom *et al.* (1985) and Muto *et al.* (1985), in preliminary reports, have examined the consequences of adrenalectomy and acute DOCA replacement (3–18 hr) on cell membrane equivalent ionic currents and conductances in the rabbit collecting tubule. These investigators report that adrenalectomy reduced both the luminal membrane conductances to sodium and potassium and depressed the equivalent sodium and potassium currents across this membrane. DOCA increase the equivalent sodium and potassium current across the luminal membrane when measured 3 hr after intramuscular injection of the hormone. Eighteen hours after DOCA treatment the basolateral membrane voltage hyperpolarized, suggesting an increase in the electrogenicity of the Na pump. Pretreatment with amiloride partially blocked the action of DOCA on luminal membrane conductances and completely inhibited the hyperpolarization of the basolateral membrane voltage. This suggests that the hormonally induced changes in membrane conductances and ATPase activity may occur by different pathways.

Muto *et al.* (1985) have also shown that in adrenalectomized rabbits receiving no hormone therapy, a high potassium diet stimulates the luminal membrane conductance to both potassium and sodium and also increases the basolateral membrane voltage, possibly by enhancing the activity of Na^+,K^+-ATPase. As discussed above these observations suggest that potassium per se may enhance sodium and potassium transport and Na^+,K^+-ATPase activity.

Additional information is required, however, to elucidate completely the mechanisms of both acute and chronic mineralocorticoid stimulation of salt transport. First, measurements of intracellular activities of sodium and potassium are required to define completely the electrochemical driving forces and ionic fluxes through conductive pathways across each membrane. Second, since supraphysiological doses of DOCA (which has both mineralocorticoid and glucocorticoid activity) have been used in many of these electrophysiological

studies, it is possible that some of the observed effects could be related to the glucocorticoid properties of the hormone. Thus, data are required from studies using more physiological levels of mineralocorticoids. Third, the nature of the cellular pathways mediating the events at the luminal and basolateral membrane remain to be elucidated.

3. Effects of Glucocorticoids

A large body of data also suggest that steroids with predominant glucocorticoid activity stimulate Na^+,K^+-ATPase activity (Charney *et al.*, 1974; Rodriguez *et al.*, 1981; Rayson and Lowther, 1984; Garg *et al.*, 1985) and augment both potassium excretion (Bartler and Fourman, 1962; Bia *et al.*, 1982; Field *et al.*, 1984; Stanton *et al.*, 1985b) and the area of the basolateral membrane of principal cells in the collecting duct (Wade *et al.*, 1979). On the basis of these observations, some investigators have considered that glucocorticoids may regulate renal potassium excretion (Bia and DeFronzo, 1982; Charney *et al.*, 1974; Rodriguez *et al.*, 1981).

These effects of glucocorticoids in most cases do not appear to be mediated specifically through glucocorticoid receptors. In general, the effects can be attributed to nonspecific or indirect effects of the hormone through binding to mineralocorticoid receptors. For example, pharmacological doses of glucocorticoids induce a kaliuresis and stimulate the activity of Na^+,K^+-ATPase in renal homogenates (Bia and DeFronzo, 1982; Charney *et al.*, 1974; Rodriguez *et al.*, 1981). Lower doses of these steroids, however, have been shown either to have no effect on potassium transport (Schwartz and Burg, 1978; Field *et al.*, 1984) and Na^+,K^+-ATPase activity (Garg *et al.*, 1985; Mujais *et al.*, 1984b; O'Neil and Hayhurst, 1985b; Petty *et al.*, 1981; Rayson and Lowther, 1984) in isolated tubule segments. If the enzyme was stimulated by glucocorticoids, the response was inhibited by spironolactone indicating that the glucocorticoid was binding to the mineralocorticoid receptor (El Mernssi and Doucet, 1983). Although glucocorticoid receptors are present in segments involved in net potassium transport, a direct effect on potassium transport has not been demonstrated in either the initial (Field *et al.*, 1984) or cortical collecting tubule (Schwartz and Burg, 1978). Furthermore, as noted above, although dexamethasone stimulates urinary potassium excretion, if the flow rate of tubular fluid is controlled by experimental design or by perfusion of single distal and collecting tubules the hormone is without effect on potassium transport (Schwartz and Burg, 1978; Field *et al.*, 1985; Stanton *et al.*, 1985b). Thus, glucocorticoids induce a kaliuresis indirectly by enhancing urine flow rate (Khuri *et al.*, 1975; Kunau and Whinnery, 1978; Good and Wright, 1979).

Although glucocorticoid levels are not enhanced in potassium-adapted animals, this class of steroid plays a critical role in the maintenance of potassium

balance. For example, when glucocorticoid levels are low, glomerular filtration rate is reduced (Wiederholt *et al.*, 1972; Pert and Pessina, 1975; Martin *et al.*, 1983; Stanton *et al.*, 1985b), often to levels that result in a decrease in tubule flow rate and sodium concentration in the distal and collecting tubules. This, in turn, sharply inhibits potassium excretion, even in the presence of high circulating levels of aldosterone (Berliner, 1961; Giebisch, 1978; Stanton and Giebisch, 1982). Furthermore by decreasing the water permeability of the cortical collecting tubule (Wiederholt and Wiederholt, 1968) glucocorticoids serve to maintain tubule flow rate in more distal segments thereby promoting potassium excretion, especially when the collecting tubule cells are stimulated by aldosterone (Field and Giebisch, 1985).

C. Chronology of Potassium Adaptation

It has long been known that both experimental animals and humans are capable of maintaining potassium balance even during the first day of treatment with a potassium-rich diet (Adam and Dawborn, 1968; Miller *et al.*, 1975; Rabinowitz *et al.*, 1984). This suggests that the mechanisms regulating potassium homeostasis have a relatively short response time. Most studies, however, have examined the mechanism of K adaptation after 10 or more days of treatment. Recent attention has been drawn to the chronology of K adaptation and to the mechanisms regulating potassium balance during acute increases in body potassium content.

To define some of the regulatory mechanisms involved in the early phase of potassium balance, we have recently performed a series of experiments in adrenalectomized rats which were studied after 10 days of basal replacement doses of both aldosterone and dexamethasone. Using this model as a baseline, the effect of acute infusions (2–4 hr) of aldosterone, dexamethasone, and potassium could be examined independently of each other. To evaluate direct effects on the function of the distal tubule in the absence of secondary alterations in luminal fluid composition and flow rate, potassium secretion in the distal tubule was measured by microperfusion at a constant rate *in situ*, while at the same time we collected the urine to measure changes in renal excretion. The major results are shown in Fig. 13. First, upon a constant background of hormone, acute KCl infusion sharply increased potassium secretion by the distal tubule and urinary potassium excretion, as well as increasing urinary flow rate. Second, we examined the effects of a selective increase in aldosterone levels that would be produced by acute infusion of KCl in adrenal intact animals. It is clear from inspection of Fig. 13 that aldosterone stimulated potassium secretion in the distal tubule; however, urinary potassium excretion did not increase. Since urine flow rate was reduced by aldosterone secondary to its antinatriuretic effect, it is likely

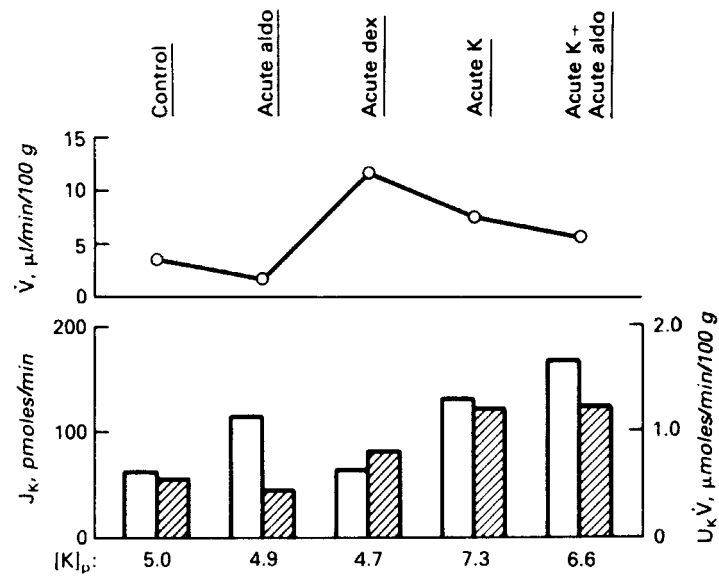

FIG. 13. Influence of adrenal corticosteroids on renal function. All animals were adrenalectomized and given basal replacement doses of aldosterone and dexamethasone for 10 days prior to study (same dose as Fig. 12), at which time they received one of five treatments shown. Abbreviations: $\dot{V}$, urine flow rate; $U_K\dot{V}$, potassium excretion (hatched bars); J_K, potassium secretion by distal tubules microperfused *in situ* (open bars); $[K]_p$, plasma potassium concentration (m*M*). [From Field and Giebisch (1985); data from Field *et al.* (1985).]

that this change may have opposed in free-flow conditions the hormone's direct effect on potassium secretion. Similar results were observed when aldosterone was infused with KCl. Whereas hyperkalemia was a potent stimulus to both potassium secretion in perfused distal tubules and urinary potassium excretion, the addition of aldosterone enhanced potassium transport only at the level of the distal tubule. Urinary potassium excretion was again unchanged by aldosterone.

Thus, the maximal rates of excretion of an acute potassium infusion do not appear to require an accompanying elevation in aldosterone levels, but rather they are mediated by hyperkalemia per se and by an increase in tubule fluid flow rate (Fig. 13). Similar observations have been made by Young (1982) and Goldstein *et al.* (1985) who found that increased urinary potassium excretion during ingestion of a high potassium diet was dependent upon natriuresis, diuresis, and hyperkalemia. The surge in aldosterone levels is significant, however, inasmuch as the rise in plasma potassium concentration was attenuated with aldosterone, thereby improving the animal's tolerance to the infusion of KCl. This effect of aldosterone is due to enhanced potassium uptake by extrarenal tissues (Bia and DeFronzo, 1981).

In concert with these studies, we have also examined the selective effects of chronic aldosterone treatment on the time course of the proliferation of basolateral membrane area of principal cells in the cortical collecting tubule. A change in basolateral membrane area was used as an indicator for the development of renal adaptation (i.e., the enhanced ability to excrete an acute KCl infusion and hence elevate potassium secretion by the collecting tubule). Results of our preliminary morphometric study (Stanton *et al.*, 1985c) are illustrated in Fig. 14. Rats were adrenalectomized and given aldosterone by implantable osmotic minipump. Plasma aldosterone levels increased by fourfold, to values measured in K-adapted animals. After 6 hr, aldosterone had no effect on basolateral membrane area; however, as noted above, potassium secretion by the late distal tubule (initial collecting tubule) increased (Field *et al.*, 1985). Na^+,K^+-ATPase activity was also not increased after a similar exposure to aldosterone in rabbits with intact adrenal glands (Hayhurst and O'Neil, 1986). In contrast, after 24 hr basolateral membrane surface density of principal cells increased by 12%, and after 3 days had increased significantly by 50%, which was slightly less than observed in K-adapted rats (Stanton *et al.*, 1981). Thus, the aldosterone-induced structural response is manifest at some time between the first and third day of exposure. Thus, the initial acute (6 hr to 1 day) increase in potassium excretion does not require an increase in Na^+,K^+-ATPase. Mujais *et al.* (1984a, 1985) and Chekal *et al.* (1986) have shown that the increase in Na^+,K^+-ATPase activity following aldosterone or a high potassium diet is dependent upon both the length of exposure

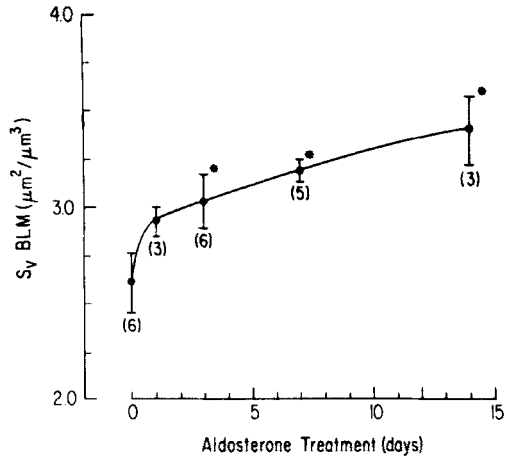

FIG. 14. Time course of aldosterone-induced increase in the surface density of the basolateral membrane of principal cells in the cortical collecting duct. All animals were given a high dose of aldosterone (2.0 μg/100 g body weight/day) and a replacement dose of dexamethasone (1.2 μg/100 g body weight) by osmotic minipump. [From Stanton *et al.* (1985c).]

and the amount of aldosterone or potassium given. Thus, if large amounts of potassium or aldosterone are given, the activity of Na^+,K^+-ATPase will increase faster compared with lower doses of these agents. This key observation may explain, in part, why there is some disagreement in the literature concerning the time course of the augmentation of Na^+,K^+-ATPase activity and electrolyte transport by mineralocorticoids.

The data of O'Neil and Hayhurst (1985b) are also in general agreement with the results summarized in Fig. 14. Na^+,K^+-ATPase activity in the cortical collecting tubule from adrenal-intact rabbits increased after a delay of 1 day following DOCA treatment. Although several investigators have shown that aldosterone increases Na^+,K^+-ATPase activity within 3 hr, it is important to note that to date this effect has only been demonstrated in adrenalectomized hormone-deficient animals and is not observed in animals with basal hormone levels (Mujais *et al.*, 1984c, 1985; Petty *et al.*, 1981; Rayson and Lowther, 1984; El Mernissi and Doucet, 1983). Since the number of aldosterone receptors increases severalfold in adrenalectomized, hormone-deficient animals (Claire *et al.*, 1981), it is highly likely that the time course of the aldosterone-induced increase in ATPase activity and ion transport may be different in adrenalectomized animals in comparison with hormone-replete animals. Consistent with these observations O'Neil and co-workers (O'Neil and Helman, 1977; O'Neil and Hayhurst, 1985b; Sansom and O'Neil, 1985) demonstrate that sodium reabsorption by the collecting tubule is stimulated by DOCA after a delay of 1 day in adrenal-intact rabbits. The conductive movement of sodium across the luminal cell membrane increases, which depolarizes the luminal membrane as more sodium enters the cell. This augments the electrochemical gradient favoring potassium secretion. The amount of Na^+,K^+-ATpase in the basolateral membrane does not increase at this time (O'Neil and Hayhurst, 1985b). At 3 days, however, the activity of the sodium pump is stimulated as determined by biochemical (O'Neil and Hayhurst, 1985b) and electrophysiological techniques (Sansom and O'Neil, 1985). Furthermore, as shown previously (O'Neil and Helmann, 1977; Stanton *et al.*, 1981; Stanton and Giebisch, 1982; and see Fig. 3), with prolonged exposure to mineralocorticoids, there is a secondary increase in net potassium transport which is the result of an increase in the potassium conductance of the luminal membrane and in the activity of Na^+,K^+-ATPase.

D. Chronic Renal Failure

Several animal models of reduced renal mass reveal that potassium balance can be maintained even when the number of nephrons is reduced dramatically (Bank and Aynedjian, 1973; Bengele *et al.*, 1978; Bourgoignie *et al.*, 1981; Epstein *et al.*, 1978; Kunau and Whinnery, 1978; Fine *et al.*, 1979, 1985;

Hayslett, 1979; Hayslett *et al.*, 1979; Schon *et al.*, 1974; Schultze *et al.*, 1971; Zalups *et al.*, 1985). This adaptive response by the remnant nephrons is largely due to increased secretion of potassium ions by the initial and cortical collecting tubule (Bank and Aynedjian, 1973; Bengele *et al.*, 1978; Kunau and Whinnery, 1978; Fine *et al.*, 1979), although the more proximal nephron segments may also be involved inasmuch as the fractional delivery of potassium into the early distal tubule is augmented. Although there is some disagreement, the ability to excrete a KCl challenge is usually attenuated compared with normal animals (Bourgoignie *et al.*, 1981; Schultze *et al.*, 1971; Schon *et al.*, 1974).

Hayslett and associates (1968, 1979) have demonstrated that the remnant nephrons undergo general hypertrophy and hyperplasia, and several investigators have shown that hypertrophy is associated with an increase in Na^+,K^+-ATPase activity in both proximal and distal nephron segments (Epstein *et al.*, 1978; Fine *et al.*, 1979; Hayslett, 1979; Hayslett and Binder, 1982; Scherzer *et al.*, 1985). We recently examined the ultrastructure of the distal nephron following 75% reduction of renal mass to determine whether specific changes in membrane area are associated with enhanced potassium secretion (Zalups *et al.*, 1985). Rats were nephrectomized and after 10 days the ultrastructures of distal convoluted cells and principal and intercalated cells were examined by morphometric techniques. Three-quarters nephrectomy produced a dramatic and specific increase in the basolateral membrane area of principal cells an effect that correlated with enhanced potassium excretion (Fig. 15). These adaptive changes in the ultrastructure of principal cells and of Na^+,K^+-ATPase are similar to those observed following long-term treatment with mineralocorticoids and chronic ingestion of a high potassium diet. Basolateral membrane proliferation was not found in intercalated cells of the initial collecting tubule or in the cells of the distal covoluted tubule.

Although the stimuli responsible for the generalized hypertrophy and hyperplasia in the collecting tubule have not been elucidated, several possibilities exist. The role of aldosterone is controversial; some investigators report that aldosterone levels are not affected by nephrectomy whereas others find a small but significant increase (reviewed by Hayslett, 1979; Smith *et al.*, 1985). Smith *et al.* (1985) have pointed out, however, that in the subset of patients with chronic renal failure whose glomerular filtration rate is less than 50% of normal, plasma aldosterone levels are elevated. Consistent with this observation we have found that when glomerular filtration rate fell by 60% following nephrectomy (Zalups *et al.*, 1985), plasma aldosterone levels increased by 80% from 20.4 ng/dl to 37.7 ng/dl (B. Stanton, J. Wade, and G. Giebisch, unpublished observations). Therefore, it is possible that aldosterone mediated the increase in basolateral membrane area, and potassium secretion by the initial and cortical collecting duct (Zalups *et al.*, 1985). In contrast, when glomerular filtration rate falls only moderately during chronic renal failure, plasma aldosterone is usually unchanged

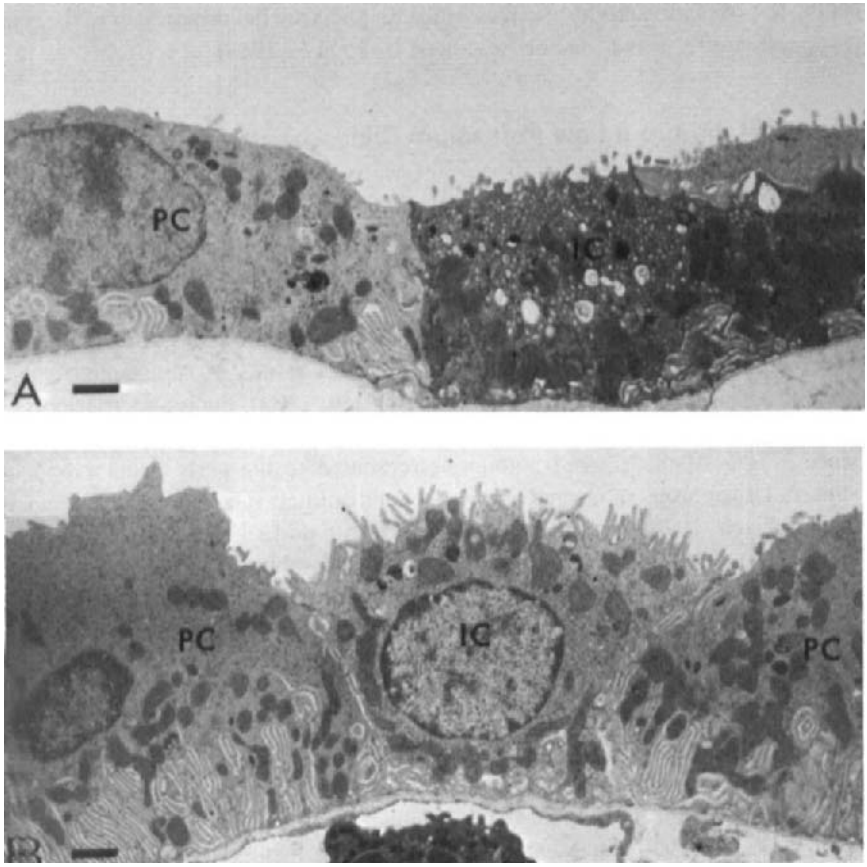

FIG. 15. Electron micrographs of initial collecting tubule in (A) control and (B) 10 days after nephrectomy (~75%) in rats. Abbreviations: PC, principal cell; IC, intercalated cell. Bar = 1 μm. [From Zalups *et al.* (1985).]

(Smith *et al.*, 1985), but plasma potassium is often elevated. In this subset of patients perhaps hyperkalemia per se leads to proliferation of Na^+,K^+-ATPase and basolateral membrane area of principal cells during chronic renal failure as it does in adrenalectomized animals with intact kidneys (Stanton *et al.*, 1986; Haystett and Binder, 1982).

These adaptive changes in the collecting tubule may also be the result of an increase in the single nephron glomerular filtration rate which has been shown to increase Na–H exchange and Na^+,K^+-ATPase in the proximal tubule (Fine and Bradley, 1985). Alternatively, or in addition, adaptation may be dependent upon alterations in the affinity of aldosterone receptors (Fine and Bradley, 1985; Zalups *et al.*, 1985), to increased excretion of other hormones known to regulate

Na^+,K^+-ATPase activity, such as prolactin and growth hormone, or to the renal production of a growth factor (reviewed by Preuss, 1984).

E. Adaptation to a Low Potassium Diet

Many of the regulatory processes involved in maintaining potassium balance are also active during adaptation to a low potassium diet. We shall now consider mechanisms that modulate K transport and tubular morphology during the development of K depletion.

Removal of potassium from the diet leads to a prompt and dramatic fall in potassium excretion (Malnic *et al.*, 1964; Linas *et al.*, 1979; Stanton *et al.*, 1981; Stetson *et al.*, 1980; Backman and Hayslett, 1983). Potassium transport by the proximal tubule and loop of Henle is not influenced by potassium depletion, since ~10% of the filtered amount is presented to the early distal tubule of superficial nephrons in normal and K-depleted animals (Giebisch, 1978; Stanton and Giebisch, 1978; Wright, 1977). In contrast to normal or K-adapted animals, there is no net potassium secretion in the distal tubule (Malnic *et al.*, 1964; Stanton *et al.*, 1981). Net potassium reabsorption by the collecting ducts has been inferred from micropuncture experiments where the fractional delivery of potassium between the late distal tubule and urine decreases (Malnic *et al.*, 1964). Microcatherization experiments have also indicated that potassium is absorbed in the medullary collecting duct in potassium-depleted rats (Backman and Hayslett, 1983). Despite absorption by the collecting duct, potassium is continually excreted in the urine (~3–5% of the filtered amount), which leads to negative potassium balance and hypokalemia.

Linas *et al.* (1979) have examined some of the factors contributing to renal potassium conservation during K depletion. They found that the decline in excretion in the first 24 hr was due to a reduction in plasma aldosterone levels, since DOCA treatment coincident with the low potassium diet inhibited the reduction in excretion. The potassium permeability of the entire distal nephron was similar to control animals during this 24-hr period. After 72 hr of K depletion, excretion was independent of mineralocorticoids and was accompanied by a reduction in the permeability of the distal nephron to potassium and a decrease in intracellular potassium content (Linas *et al.*, 1979). Declines in both parameters would be expected to reduce the secretory flux from cell to lumen, leaving the active reabsorptive potassium transport system unopposed (see Fig. 4).

Several groups of investigators have reported a relationship between dietary K depletion and the structure of intercalated cells in the medullary collecting duct (Oliver *et al.*, 1957; Toback *et al.*, 1976; Evan *et al.*, 1980; Stetson *et al.*, 1980). In an early light microscope study, Oliver *et al.* (1957) proposed that the intercalated cell population was labile and that the incidence of these cells increased

after K depletion. These observations were later confirmed with light (Toback *et al.*, 1976) and electron microscopy (Evan *et al.*, 1980). In contrast, others have reported that there is no change in the number of intercalated cells with K depletion (Hansen *et al.*, 1980; Stetson *et al.*, 1980; Stanton *et al.*, 1981). This controversey is still not resolved. All investigators, however, have noted an increase in the luminal membrane area of intercalated cells in the medullary collecting duct following K depletion (Oliver *et al.*, 1957; Toback *et al.*, 1976; Evan *et al.*, 1980; Stetson *et al.*, 1980) and have suggested that this cell type may reabsorb potassium based on the correlation between increases in potassium reabsorption and amplification of the luminal cell membrane.

In a detailed study, Stetson and co-workers (1980) observed that there are two populations of intercalated cells based on the distribution of rod-shaped particles in the luminal membrane. As originally described by Humbert *et al.* (1975), only the intercalated cell type in the collecting duct have rod-shaped intramembranous particles (the particles in the luminal cell membrane which are observed by freeze-fracture techniques appear to be associated with "studs" in the membrane, observed by transmission electron microscopy). According to Stetson *et al.* (1980), one population of cells with the characteristics of intercalated cells by transmission electron microscopy does not contain a high-density of rod-shaped particles in the luminal membrane, but does have numerous vesicles in the apical cytoplasm. The second type of intercalated cell has a high density of rod-shaped particles in the luminal membrane and has only a few cytoplasmic vesicles. Both cell types have high-density intramembrane particles in the vesicles in the apical cytoplasm. K depletion produces a shift in the intercalated cell population such that all intercalated cells have a high density of luminal membrane rod-shaped particles (Fig. 16). Furthermore, K depletion is associated with a threefold increase in the luminal membrane surface area of intercalated cells and a sharp decrease in the number of vesicles with rod-shaped particles in the apical cytoplasm. On the basis of these observations, Stetson *et al.* (1980) suggested the possibility that K depletion induces the fusion of vesicles with the luminal membrane, contributing both membrane and rod-shaped particles to the luminal domain. This suggests that the rod-shaped particles mediate potassium reabsorption. As shown in Fig. 16, membrane structure and basolateral membrane area in principal cells were not affected by K depletion. However, it should be stressed that the change in membrane structure with K depletion could be unrelated to alterations in cellular potassium transport. Indeed, potassium reabsorption in the outer medullary collecting duct could be paracellular inasmuch as this pathway is permeable to potassium and the positive-lumen voltage would favor reabsorption (Koppen, 1985, 1986). Furthermore, K depletion stimulates paracellular to potassium permeability which would also augment paracellular reabsorption (Wingo, 1986).

Because K depletion is also associated with an increase in H^+ ion secretion by

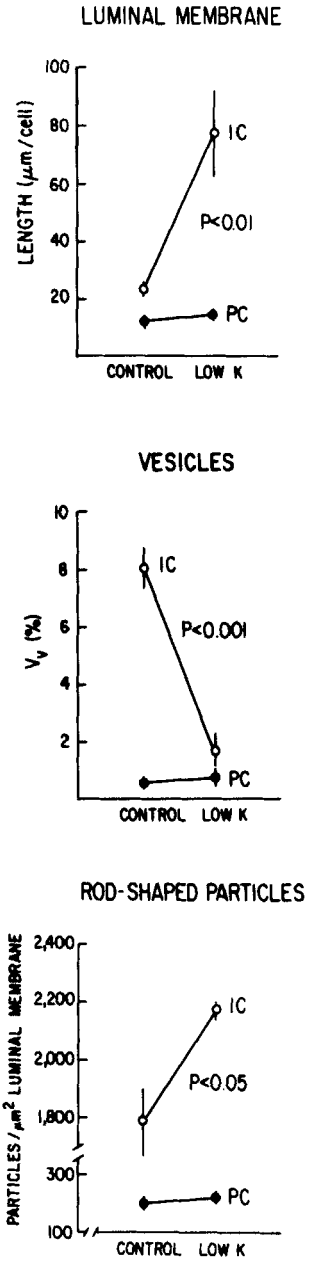

FIG. 16. Ultrastructural changes in intercalated and principal cells of medullary collecting duct after 4 weeks of a low K diet. Abbreviations: IC, intercalated cell; PC, principal cell; V_V, ratio of volume of apical vesicles to apical cytoplasm. [Data from Stetson *et al.* (1980).]

the medullary collecting duct, a process that is believed to be electrogenic (Giebisch, 1978; Stetson *et al.*, 1980; Wright, 1977; Stanton and Giebisch, 1982; Koeppen, 1985), it has also been suggested that the rod-shaped particles in intercalated cells may be the transport protein involved in the secretion of hydrogen. Indeed, Stetson and Steinmetz (1984) point out that rod-shaped intramembranous particles are present in all examined epithelial cells that secrete hydrogen by an electrogenic process. Thus, it is possible that the changes in intercalated cell structure during K depletion, which enhances urinary acidification, is related to increased hydrogen ion secretion.

Madsen and Tisher (1983, 1984) have also shown that stimulation of hydrogen ion secretion in the outer medullary collecting duct by either respiratory or metabolic acidosis is associated with an increase in the area of the luminal membrane and a decrease in the area of the luminal membrane and a decrease in the number of vesicles in the cytoplasm of intercalated cells. They also suggested that the membrane of the vesicles contains proton pumps and that hydrogen ion secretion is stimulated following insertion into the luminal membrane of new pumps. Because potassium reabsorption and hydrogen ion secretion by the collecting tubule increase parri passu, it has been attractive to speculate that hydrogen ion secretion and potassium absorption across the luminal cell membrane are coupled (Stanton and Giebisch, 1981; Stetson *et al.*, 1980). Considering the electrochemical gradients across the luminal membrane hydrogen–potassium countertransport, if it exists, must be a primary active transport process.

The insertion of new membrane and transport proteins into the luminal cell membrane of intercalated cells induced by acidosis or potassium depletion may occur relatively quickly compared with the aldosterone-induced proliferation in membrane area in principal cells. Waack *et al.* (1985) have recently shown that a reduction in potassium in the incubation media of cultured cells from the medulla of the monkey kidney leads to a dramatic increase in luminal membrane area in only 3 min. Furthermore, the membrane proliferation correlates with a sharp increase in the uptake of several metabolic substrates from the media into the cell. Thus, the ultrastructural response of intercalated cells, which is associated with enhanced transport, may occur considerably more rapidly than the response of principal cells during K adaptation.

IV. INTEGRATIVE CELL MODEL

Taken together, the experimental results described above may be assembled into the following somewhat speculative cell model to account for the accelerated rate of potassium secretion by the collecting tubule during K adaptation.

When challenged with increased potassium, enhanced potassium secretion by the collecting tubule can be divided into two phases: acute and chronic. The

acute effect is likely the result of at least three identifiable factors. First, hyperkalemia inhibits sodium and fluid reabsorption in the proximal tubule (Brandis *et al.*, 1972), increasing the delivery of fluid into the distal and collecting tubule, which augments potassium secretion (Khuri *et al.*, 1975; Wright *et al.*, 1971; Kunau *et al.*, 1974; Good and Wright, 1979). Second, hyperkalemia per se stimulates potassium secretion by the collecting tubule, most likely by increasing intracellular potassium activity (Oberleithner and Giebisch, 1981), and depolarizing the basolateral and luminal membrane voltage which would increase the electrochemical gradient favoring potassium secretion (Koeppen *et al.*, 1983; O'Neil and Sansom, 1984a,b). Since the potassium conductance of the luminal membrane is voltage dependent and depolarization increases conductance, this would also augment potassium secretion (Hunter *et al.*, 1984; Koeppen *et al.*, 1984; Palmer and Frindt, 1985). Finally, hyperkalemia stimulates aldosterone secretion which after a 1- to 2-hr latent period, augments potassium secretion, at least at the level of the initial collecting tubule when tubule flow is constant (Field *et al.*, 1984). As shown by Sansom *et al.* (1985), the acute elevation in aldosterone increases the sodium conductance of the luminal membrane which, by depolarizing this barrier, increases the electrochemical driving force for potassium secretion (Fig. 17). As a result of the increase in luminal membrane sodium conductance, sodium entry rises, thereby augmenting the turnover rate of Na^+,K^+-ATPase. This latter event serves to maintain potassium uptake across the basolateral membrane. The total number of Na^+,K^+-ATPase units is not elevated at this time.

The effects of aldosterone on the urinary bladder are also consistent with the early effect of mineralocorticoids on the sodium entry step (Park and Edelman, 1984a; Gerring *et al.*, 1982). In both toad (Park and Edelman, 1984a,b; Gerring *et al.*, 1982) and rabbit bladder (Lewis and Wills, 1983) aldosterone increases the conductance of the luminal membrane to sodium without an effect on the K_m (Park and Edelman, 1984a) or V_{max} of Na^+,K^+-ATPase (Park and Edelman, 1984a,b; Lewis and Wills, 1983). Garty and Edelman (1983) have demonstrated convincingly that the aldosterone-induced increase in luminal membrane sodium conductance is due to activation of preexisting nonconducting channels to an active form. Again, these results support the concept that the initial effect of aldosterone is on the sodium permeability of the luminal membrane.

After exposure to a high potassium diet for 1–3 days, the collecting tubule adapts and increases the ability to secrete potassium in the following manner (Fig. 17). The sustained increase in aldosterone levels and potassium intake both lead to an increase in the amount of Na^+,K^+-ATPase in the basolateral membrane and to a parallel and concomitant increase in the conductance of the luminal membrane of principal cells to potassium. Both events augment potassium secretion. Furthermore, the hyperpolarization of the basolateral mem-

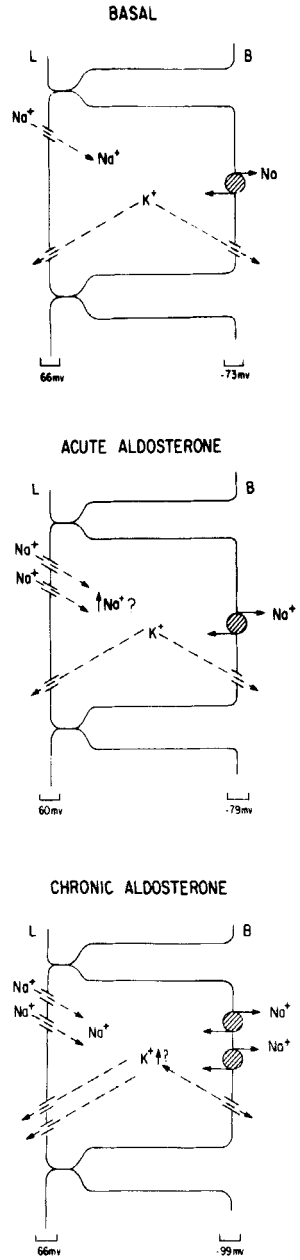

FIG. 17. Cell schema of aldosterone-induced stimulation of Na^+ and K^+ transport by principal cells in the collecting tubule. The acute effects (~2 hr to 1 day) include an increase in the Na^+ conductance of the luminal membrane and a depolarization of the luminal membrane voltage. Chronic effects (1–7 days) include a parallel increase in the K conductance of the luminal membrane and in the number of Na^+,K^+-ATPase units. [Numbers below each cell depict measured membrane voltages from Sansom and O'Neil (1985).] The electroneutral KCl cotransport system shown in Fig. 4 is not reproduced to simplify the drawing. For more details, consult the text.

brane (Koeppen *et al.*, 1983; Sansom and O'Neil, 1985) may favor uptake of potassium into the cell, resulting in an increase in intracellular potassium activity which would also stimulate potassium secretion (Oberleithner and Giebisch, 1981).

V. SUMMARY

Renal K adaptation can be induced by both an increase in potassium intake and plasma aldosterone levels and results in an enhanced capacity to excrete potassium during K infusion, primarily due to accelerated secretion by principal cells in the collecting tubule. Adaptation requires 1–3 days to develop and is associated with an increase in the amount of Na^+,K^+-ATPase in the basolateral membrane of principal cells. Furthermore, enhanced potassium secretion is also mediated by an increase in potassium conductance of the luminal cell membrane.

Adaptation allows the organism to restore whole-body potassium balance more rapidly than normal and also attenuates the hyperkalemia following potassium loading. Furthermore, adaptation increases the flexibility of an organism in its response to alterations in potassium balance, which enables survival of otherwise life-threatening hyperkalemia.

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Chapter 12

Quantitative Analysis of Steady-State Potassium Regulation

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I. INTRODUCTION

Potassium transfer across cell membranes in the kidney and in all cells of the body is coordinated by a complex set of determinants in order to precisely regulate intra- and extracellular potassium concentrations and to maintain constant potassium balance. While our comprehension of the mechanisms responsible for the membrane and tissue processes has improved, intriguing questions remain concerning integration of these functions into a system which responds to a variety of challenges. Analysis of such a system using designs traditionally employed in biological science is generally unproductive because of the confounding effects of simultaneous operations of several interacting feedback control processes.

During the past 10 years, the potassium control system has been studied in this laboratory using a nontraditional approach. Because the system is complex in that it is composed of several in-series and parallel interacting components, the operation of the system could not be analyzed by direct experimental techniques. Instead, a hypothesis concerning the structure and function of the control system was constructed and tested for validity. The hypothesis was in the form of a mathematical model derived from a combination of new and existing concepts concerning operations of portions of the K control system. In many cases, the equations related to the concepts were directly derived from the quantitative data obtained in the experiments described in the following section. In other cases, the equations were derived from information present in the literature. The equations were arranged for solution using a digital computer (Digital Equipment, Maynard, Massachusetts). Therefore, the responses of the hypothetical system to

a wide range of challenges can be determined. The validity of the hypothesis can be tested by comparing the model responses to perturbations with the responses of the real system to the same perturbation in experiments conducted here for the purpose of testing the hypothesis, or in experiments or clinical findings described in the literature.

The hypothesis being considered here concerns the nature and function of the system that regulates K over periods of time ranging from hours to months. Therefore, no short-term, fast-acting mechanisms are included. Also, because the model is designed to investigate the functional integration of the entire control system, the functions of many subsystems have not been considered in detail. In several instances, the transfer functions that have been used to derive an output variable from an input span whole domains of active physiology. This was done only when the variables involved were of marginal importance to the subject of K regulation and the numerous physiological processes included in the transfer function were not individually affected by the operation of the K control system. These simplifying functions were used especially in the modeling of GFR and arterial pressure regulation. In reviewing the model, the reader will see that each section could be expanded into a very complex model and a career of research. However, the goal of the present study was to analyze the function of the overall control system, and therefore only the functions important to that goal were included here.

II. TENETS OF THE HYPOTHESIS

The basic concepts of the hypothesis, or tenets, are listed in Table I (Young, 1985). Each of these is a crucial part of the hypothesis and is represented in the model by groups of equations. In this section, each tenet will be discussed along with supporting information.

A.1. Redistribution of K between the Intra- and Extracellular Spaces Is a Function of Aldosterone

In any in-depth consideration of whole-body K regulation, one of the first questions to be confronted is how are changes in total body K related to changes in plasma K, or how is K distribution determined. A number of clinical and experimental observations indicate that aldosterone is importantly involved in the complex relationship between overall K balance and plasma K concentration.

For example, in a study by Pan and Young (1982), five times the normal level of aldosterone was infused into dogs for 14 days. Although there was no change

TABLE I
TENETS OF THE HYPOTHESIS[a]

1. The distribution of potassium between the intra- and extracellular spaces is a function of aldosterone concentration
2. Potassium excretion is primarily a function of plasma potassium concentration, and this function is modified by aldosterone concentration and distal nephron flow rate
3. Aldosterone concentration is primarily a function of plasma potassium concentration, and this function is modified by angiotensin II concentration
4. Distal nephron flow rate is primarily a function of GFR, and this function is modified by arterial pressure and plasma potassium concentration
5. Angiotensin concentration is a function of distal nephron flow rate
6. Arterial pressure is a function of extracellular fluid volume, and this function is modified by angiotensin II concentration
7. Sodium excretion is a function of distal nephron flow rate, and this function is modified by angiotension II concentration, aldosterone concentration, and arterial pressure

[a]See Section II,A–G.

in K excretion or intake during the infusion period, plasma K fell from 4.8 to 3.2 mEq/liter, a 33% decrease in plasma K with no apparent change in total body K.

To analyze closely the effect of aldosterone on K distribution, two groups of experiments were conducted (Young and Jackson, 1982). In the first, seven normal dogs received continuous infusion of aldosterone at a high physiological rate, 250 μg/day. Total exchangeable K (Ke) and plasma K were measured before and at 4 and 6 days after aldosterone infusion. Plasma K fell by 20%, while Ke decreased by 8% after 6 days of infusion; the ratio between extracellular and total body K had been altered by the aldosterone infusion. In the second study, 10 adrenalectomized dogs received aldosterone infusion first at 50 μg/day, then at 250 μg/day, while on each level of aldosterone infusion, three levels of K intake were given by intravenous infusions. When the animals were in electrolyte balance at each level of aldosterone and K (after at least 7 days on each level of infusion), Ke (expressed as mEq/kg) and plasma K were measured. The two variables were plotted against each other, Ke being the independent variable. Data taken while the dogs received 50 μg/day aldosterone were described by the equation, plasma $K = 0.100\ Ke + 0.55$, while those obtained at 250 μg/day were fitted by the equation, plasma $K = 0.057\ Ke + 1.30$ (Fig. 1). Data from the two groups of experiments are consistent with the hypothesis that aldosterone alters the distribution of K between the intra- and extracellular spaces, a greater portion of total K being intracellular at higher levels of aldosterone. In summary, the results of these studies suggest that aldosterone regulates K not only by affecting excretion, but also by influencing its distribution within the body.

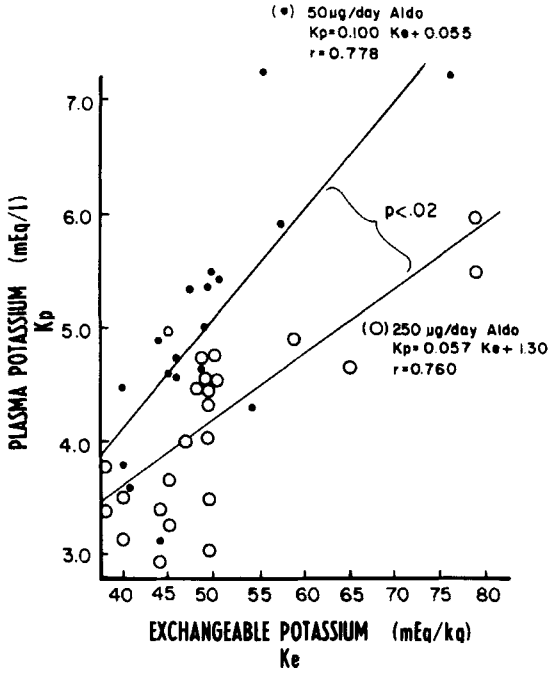

FIG. 1. The effect of aldosterone on K distribution can be seen here as the relationship between exchangeable Ka and plasma K rotated downward as the rate of aldosterone replacement is increased from normal (50 µg/day) to five times normal. [From Young and Jackson (1982).]

B.2. K Excretion Is Primarily a Function of Plasma K Concentration, and This Function Is Modified by Aldosterone and Distal Nephron Flow Rate

This portion of the hypothesis is supported by the work of others presented in previous chapters of this volume and by the data of several studies conducted in this laboratory. In order to obtain data to be used in a systems analysis of K regulation, quantitative data describing the operation of the components of the system had to be collected under uniform conditions. With this consideration in mind, experiments were conducted to analyze the relationship between plasma K concentration and steady-state renal K excretion and to determine how aldosterone and Na intake/excretion affected the relationship.

The relationship between plasma K concentration and renal K excretion was studied using seven chronically adrenalectomized dogs maintained on a constant rate of aldosterone replacement, 50 µg/day given continuously intravenously in 50 ml of saline (Young, 1982). A basal level of glucocorticoids was given in the same manner. Sodium intake was held at the normal level, 30 mEq/day, while K

intake was varied in four steps of 7- to 10-days' duration from 10 to 30 to 100 to 200 mEq/day. At the conclusion of each level of intake, plasma K and renal excretion as well as other variables known to influence K excretion were measured. There were minimal changes in arterial pH, mean arterial pressure, extracellular fluid volume, or GFR at any of the levels of K intake. The values for plasma K and renal K excretion are plotted in Fig. 2. The magnitude of the slope of the relationship between plasma K and renal excretion, 260 mEq/day per mEq/liter increase in concentration above 4.2 mEq/liter, suggests that this relationship may be the most prominent controller of K above the normal concentration of 4.0–4.2 mEq/liter. This relationship acting alone provided excellent regulation of plasma K in response to increases in K intake above normal and minimal control capability in response to subnormal intakes. These results, obtained under conditions of a fixed aldosterone concentration and Na intake, suggest that dynamic participation of the aldosterone feedback loop is not of great importance in response to above normal K intake. However, below the normal level of intake, the slope of the plasma K urinary excretion relationship is much less impressive, 11 mEq/day per mEq/liter. Apparently, over this range of

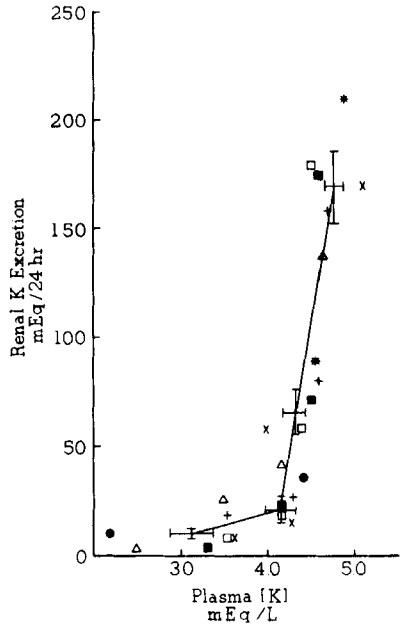

FIG. 2. Renal K excretion plotted as a function of plasma K concentration under steady-state conditions. The dogs used in this study were adrenalectomized and maintained on normal levels of aldosterone and methylprednisolone infused intravenously. [From Young (1982).]

intake, participation of the aldosterone feedback loop is necessary for accurate control of plasma K.

The effect of aldosterone on renal K excretion was evaluated using a design similar to the one just described (Young and Paulsen, 1983). Two groups of chronically adrenalectomized dogs were used. In Group I (n = 6), aldosterone was infused intravenously at a rate of 20 μg/day (0.4 times normal), while K intake was changed in steps of 7- to 10-days' duration from 10 to 30 to 100 mEq/day. At the completion of each step, plasma K, urinary K excretion, and other variables that potentially may affect renal function were measured. In group II (n = 6), a similar protocol was followed except that aldosterone was infused at 250 μg/day (five times normal), and the potassium intake levels were 30, 100, and 200 mEq/day. Data from these two groups were combined with those collected in the study described above in which 50 μg/day of aldosterone was infused; the studies were similar in protocol so the data could be combined usefully, as in Fig. 3. The increase in aldosterone infusion rate shifted the relationship between plasma K and K excretion to the left so that at a given level of plasma K, a greater amount of K was excreted. In the normal range of plasma K (4.00–4.40 mEq/liter), increasing aldosterone replacement from 0.4 to 5.0 times of normal was associated with a four- to eightfold increase in daily K

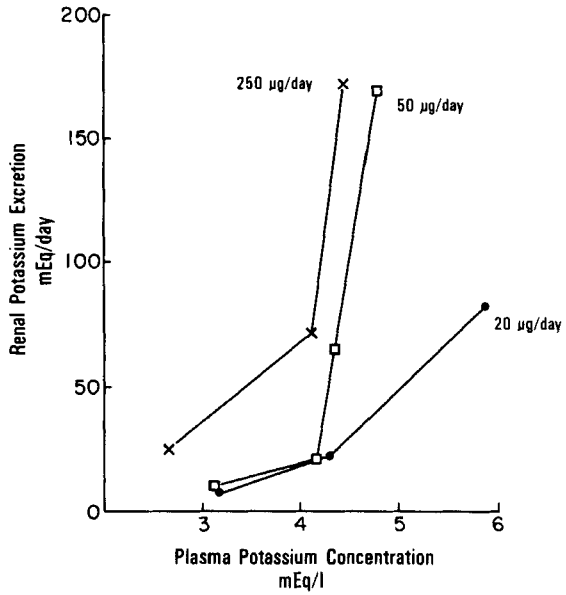

FIG. 3. The steady-state relationships between plasma K and renal K excretion at three levels of aldosterone replacement. [From Young and Paulsen (1983).]

excretion. Normalized data obtained by extrapolation from Fig. 3 are presented in three-dimensions in Fig. 4.

In spite of the fact that the data from the present study indicate that aldosterone can have important effects on renal potassium excretion in the upper part of the normal range of plasma potassium concentration, some of the information from this study together with data from previous work raise questions concerning the actual quantitative role of the hormone in control of potassium excretion in response to a potassium challenge. In an experiment conducted previously

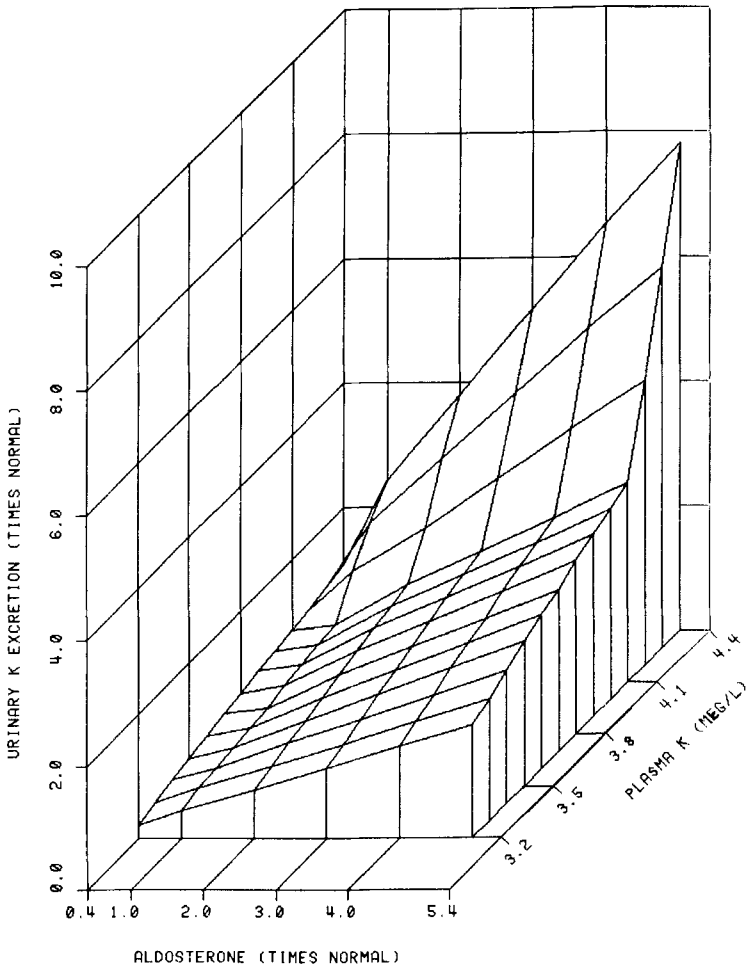

FIG. 4. Three-dimensional representation of the interaction between plasma K and aldosterone in affecting renal K excretion. The data were obtained from Fig. 3 and normalized.

(Young *et al.*, 1976a) in this laboratory under conditions similar to the present ones, increasing potassium intake of normal dogs from 30 to 200 mEq/day resulted in a steady-state increase in aldosterone concentration of 136% (from 10.6 to 23.1 ng/dl), an increase in plasma potassium concentration from 4.12 to 4.65 mEq/liter, and an increase in urinary potassium excretion from 23 to 190 mEq/day. Judging from the data of the present study, especially as displayed in Fig. 4, most of this increase in excretion resulted from movement along the potassium concentration axis, i.e., one would expect from the data presented in Fig. 4 that the 0.5 mEq/liter increase in potassium concentration would have a much greater kaliuretic effect than the 136% increase in aldosterone concentration. Apparently, when the system receives a challenge in the form of an increase in potassium intake, plasma potassium concentration rises, stimulating both aldosterone secretion and renal potassium excretion. However, the kaliuretic effect of a small rise in plasma potassium concentration combined with a small increase in aldosterone concentration readily returns the system to balance, which limits further increases in plasma potassium to levels that do not stimulate very high rates of aldosterone secretion. This appears to be the case throughout the region of the surface surrounding the normal operating point (plasma potassium = 4.2 mEq/liter, aldosterone = 1.0). Therefore, under normal operating conditions, the kaliuretic response of the system to a potassium challenge is probably mediated primarily through the effects of the increase in plasma potassium concentration.

Changes in sodium intake can affect the rate of K excretion presumably by changing the rate of flow through the distal nephron. Once again, to determine the magnitude of this effect and to obtain data to be used in the systems analysis of potassium regulation, experiments were conducted in the same standardized manner as described in the previous sections so that compatible quantitiative data could be gathered (Young *et al.*, 1984a).

Seventeen chronically adrenalectomized dogs were used. Throughout the study, aldosterone was replaced by continuous intravenous infusion at a rate of 50 μg/day, the normal rate for 20-kg dogs. Potassium intake was changed in steps of 7- to 10-days' duration from 10 to 30 to 100 mEq/day. The dogs were divided into three groups according to the level of sodium intake they received, 10, 100, and 200 mEq/day.

Plotting the steady-state relationships between plasma K and renal K excretion at the three levels of Na intake yielded a family of three function curves (Fig. 5) similar to those described previously. The data were normalized and plotted in three dimensions in Fig. 6.

Varying sodium intake within the physiological range from approximately one-third normal to three times normal appears to have a strong effect on the steady-state relationship between plasma potassium and potassium excretion. Based on the regression equations describing the potassium excretion data at the

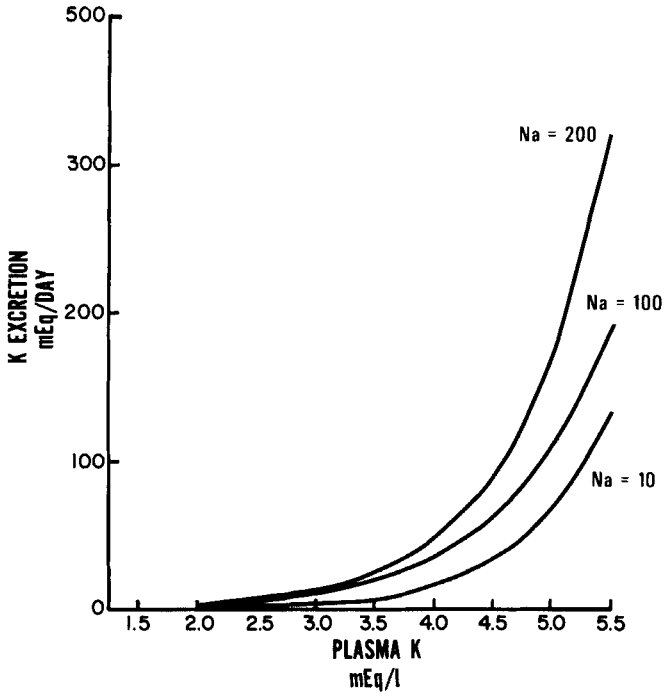

FIG. 5. Effect of Na intake on the relationship between plasma K and K excretion, increasing Na intake shifts the relationship up and to the left so that a higher rate of K excretion at a given level of plasma K would be expected as Na intake is elevated. [From Young *et al.* (1984a).]

three levels of sodium intake, one can predict that at the normal level of plasma potassium concentration, 4.0 mEq/liter, the animals would excrete potassium at a rate of 17 mEq/day at the 10 mEq/day sodium intake, 37 mEq/day at the 100 mEq/day rate of sodium intake, and 47 mEq/day at the 200 mEq/day sodium intake level. Such a powerful effect is probably the result of an increase in distal nephron and collecting duct flow rate associated with the increase in sodium intake. The increase in flow in these regions would be expected to decrease luminal potassium concentration and increase the gradient favoring movement of potassium into the lumen, as described by Khuri *et al.* (1975) and Good and Wright (1979).

In intact normal animals, changes in sodium intake within the normal range do not affect potassium excretion or perturb plasma potassium regulation, apparently due to compensatory changes in aldosterone secretion. Excellent coordination between the zona glomerulosa of the adrenal gland and the distal nephron must exist for changes in sodium intake and distal flow to be balanced exactly and promptly by changes in aldosterone secretion. Aldosterone secretion re-

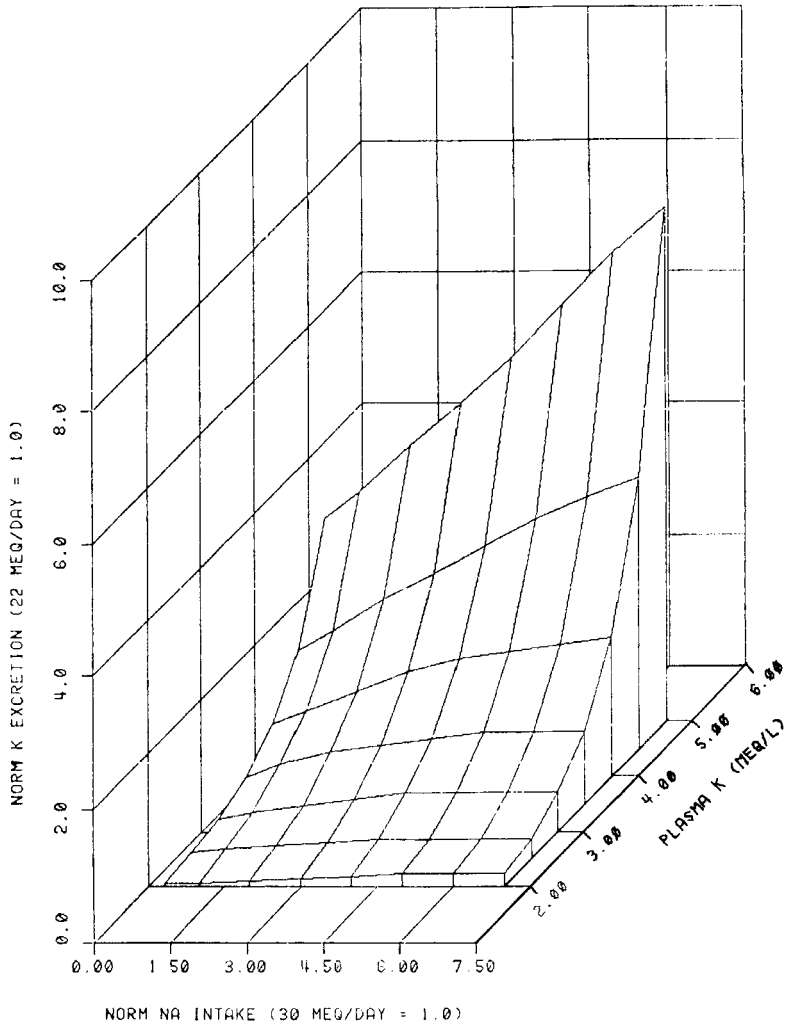

FIG. 6. Three-dimensional representation of normalized data obtained from Fig. 5, showing the combined effects of Na intake and plasma K on K excretion.

sponds in the absence of changes in plasma potassium concentration and therefore must be signaled primarily by changes in angiotensin II resulting from alterations in renin release. A sensor of distal nephron flow rate at the macula densa that affected the rate of renin release and hence the rate of aldosterone secretion could provide the excellent coordination required to account for the observed accuracy of potassium regulation during changes in sodium balance.

C.3. Aldosterone Concentration Is a Function of Plasma K Concentration, and This Function Is Modified by Angiotensin II Concentration (AII)

This portion of the hypothesis is directly supported by the results of a study designed to analyze the regulation of aldosterone concentration. To obtain the data required for this analysis, different combined levels of angiotensin II (AII) and K were continuously infused intravenously in six large dogs (22.6 ± 1.0 kg), while plasma aldosterone concentration (PAC) was monitored (Young *et al.*, 1984b). Three levels of K intake, 10 100, and 200 mEq/day, were combined with three levels of AII infusion, 0, 5, and 10 ng/kg/min. Each combined infusion regimen was maintained for 5 days and data were sampled on Days 1, 2, and 5.

The results are summarized in Figs. 7, 8, and 9 in which aldosterone con-

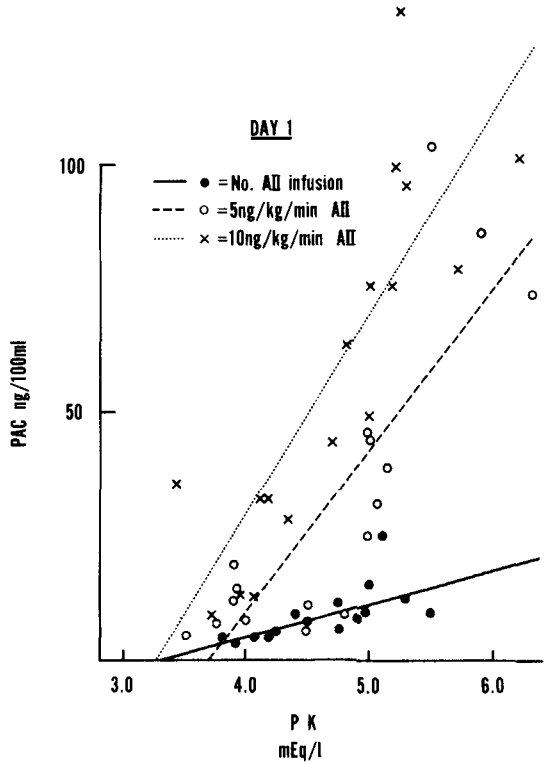

FIG. 7. Aldosterone concentration as a function of plasma K at three levels of angiotensin II infusion on the first day of infusion. [From Young *et al.* (1984b).]

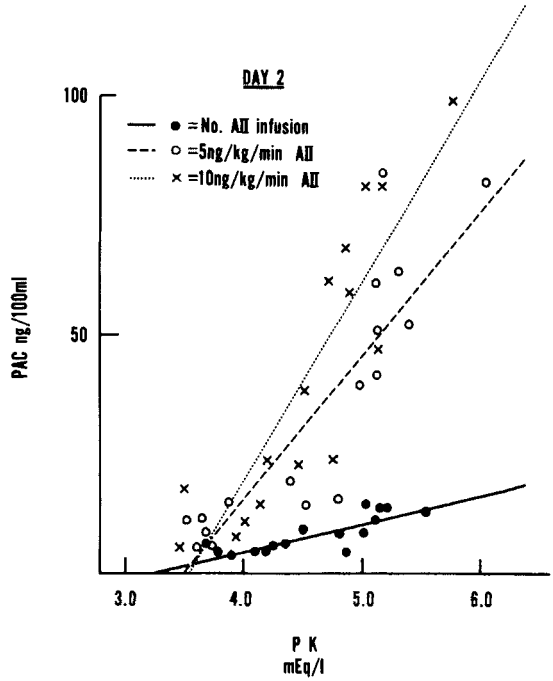

FIG. 8. Data from the second day of angiotensin II infusion, as in Fig. 7. [From Young *et al.* (1984b).]

centration is plotted versus plasma K concentration for each level of angiotensin II infusion in the 3 days of data collection. The regressions shown in the figures were obtained by the least squares technique. In each figure, notice that with increasing rates of angiotensin II infusion, the regressions rotate up and to the left without affecting the point at which the regressions reach the zero aldosterone level. This effect indicates angiotensin and plasma K are interacting multiplicatively in stimulating aldosterone. Normalized data from Day 5 are plotted in three dimensions in Fig. 10.

This multiplicative interaction between the two stimuli has some noteworthy consequences. First, if the concentration of either angiotensin II or plasma K is low, even a high level of the other will not elicit a large response in aldosterone secretion. This effect is apparent in Fig. 10. Increasing angiotensin to even five or six times normal will result in only modest increases in aldosterone concentration if plasma K is held in the 3.5 mEq/liter range. Likewise, when angiotensin II levels are fixed in the normal range, large elevations in plasma K bring about increases in aldosterone that are modest in comparison with those resulting from the same change in plasma K at higher levels of angiotensin. Furthermore, at

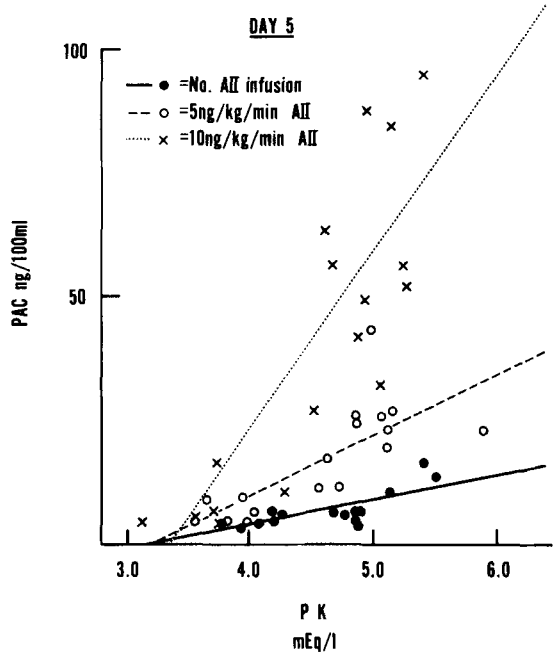

FIG. 9. Data from the fifth day of angiotensin II infusion, as in Fig. 7. [From Young *et al.* (1984b).]

high levels of angiotensin II concentration, small changes in plasma K produce very large changes in aldosterone concentration. From the information presented in Fig. 10, one could calculate that at an angiotensin level six times normal, decreasing plasma K from 4.5 to 4.0 mEq/liter would decrease plasma aldosterone concentration from ~10.0 times normal to ~5.3 times normal. From these examples and the other data presented in Fig. 10, it is clear that aldosterone regulation cannot be adequately analyzed unless the multiplicative interaction between angiotensin II and plasma K is considered.

D.4. Distal Nephron Flow Rate Is Primarily a Function of GFR, and This Function Is Modified by Arterial Pressure and Plasma K Concentration

In the model, distal nephron flow rate is taken to be a fraction of GFR. Normally, the value is 15%. Elevations of arterial pressure increase this fraction in the model, in agreement with the experimentally observed effect of renal perfusion pressure on proximal tubular reabsorption. Increases in plasma K also increase the fraction of GFR delivered to the distal nephron, which would be predicted from the data demonstrating that plasma K elevations inhibit reabsorp-

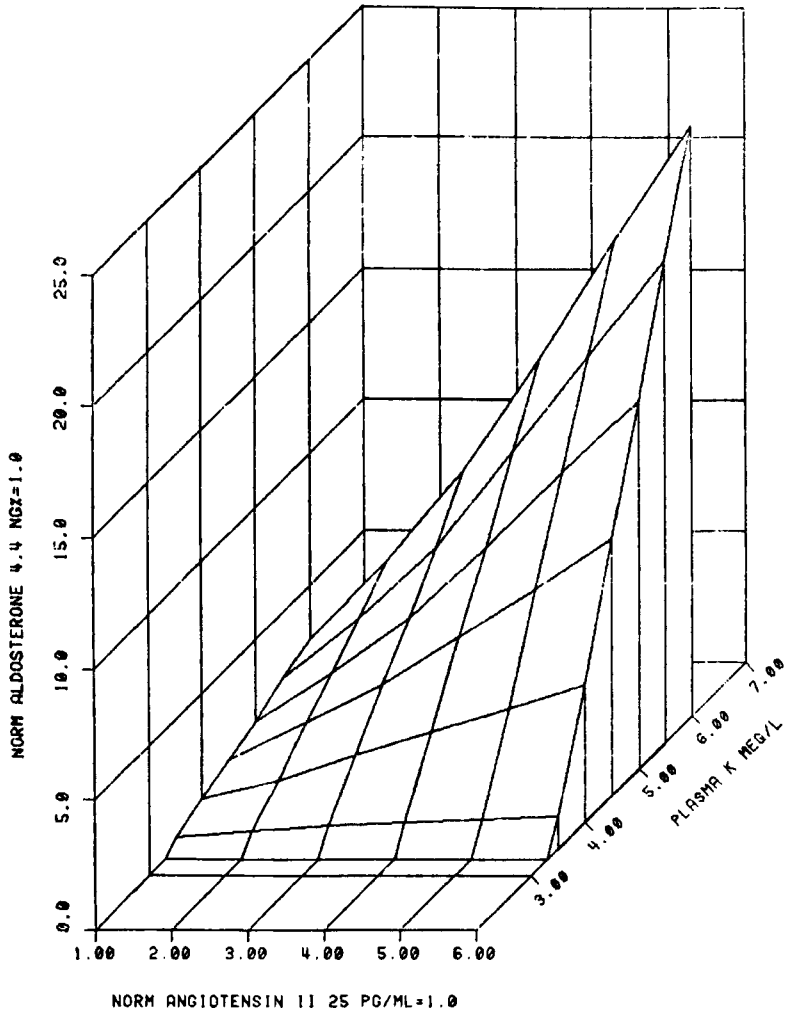

FIG. 10. Three-dimensional presentation of the interaction between normalized angiotensin II concentration and plasma K in stimulating aldosterone. The data were obtained from those presented in Fig. 9.

tion in the proximal tubular (Brandis *et al.*, 1972) and loop of Henle (Sufit and Jamison, 1983; Kirchner, 1983).

E.5. Angiotensin II Concentration Is a Function of Distal Nephron Flow Rate

This statement is derived from the macula densa hypothesis for regulation of renin release (Vander and Luciano, 1967). In the model, angiotensin levels

increase in an inverse relationship with changes in distal nephron flow. Renin release rate and angiotensin I generation and conversion, distribution, and metabolism are not modeled, since these steps would never be affected individually in the situations under study. Therefore, only the product of the final step, angiotensin II concentration, is related to the controlling variable, distal nephron flow rate.

F.6. Arterial Pressure Is a Function of Extracellular Fluid Volume, and This Function Is Modified by Angiotensin II Concentration

This statement is well supported by a great many experiments from this laboratory and others (Guyton, 1980a,b). This statement is not exclusive; i.e., it does not imply that there are not other factors that modify the relationship between extracellular fluid volume and arterial pressure. However, angiotensin II concentration is the only variable normally involved in the proposed model of K regulation which affects the relationship.

G.7. Sodium Excretion Is a Function of Distal Nephron Flow Rate, and This Function Is Modified by Angiotensin II, Aldosterone Concentration, and Arterial Pressure

The rate of Na excretion is determined from the rate of flow into the distal nephron and the rate of reabsorption of Na out of the luminal fluid. Aldosterone's effect in the distal nephron is well established. Angiotensin is also an important antinatriuretic hormone, probably more actively involved in regulation of Na excretion than is aldosterone (Hall *et al.*, 1977, 1980; Young *et al.*, 1982). The tubular actions of angiotensin have not been fully studied. However, recently it has been shown that physiological concentrations have a relatively weak effect on Na reabsorption in the proximal tubule (Schuster *et al.*, 1984), and little is known about its effect in the distal nephron. Because angiotensin is known to be of major importance in regulation of Na excretion and because its major site of action does not appear to be the proximal tubule, its effect has been hypothesized to be on the distal nephron.

III. THE MATHEMATICAL MODEL

In order to analyze and test the potassium regulation hypothesis constructed from the tenets described above, a mathematical model solvable on a digital computer devised from the tenets was constructed (Young, 1985). It is presented in Fig. 11 in graphical fashion and as mathematical equations written in FORTRAN notation in Table II. A glossary of terms is given in Table III, and a list of

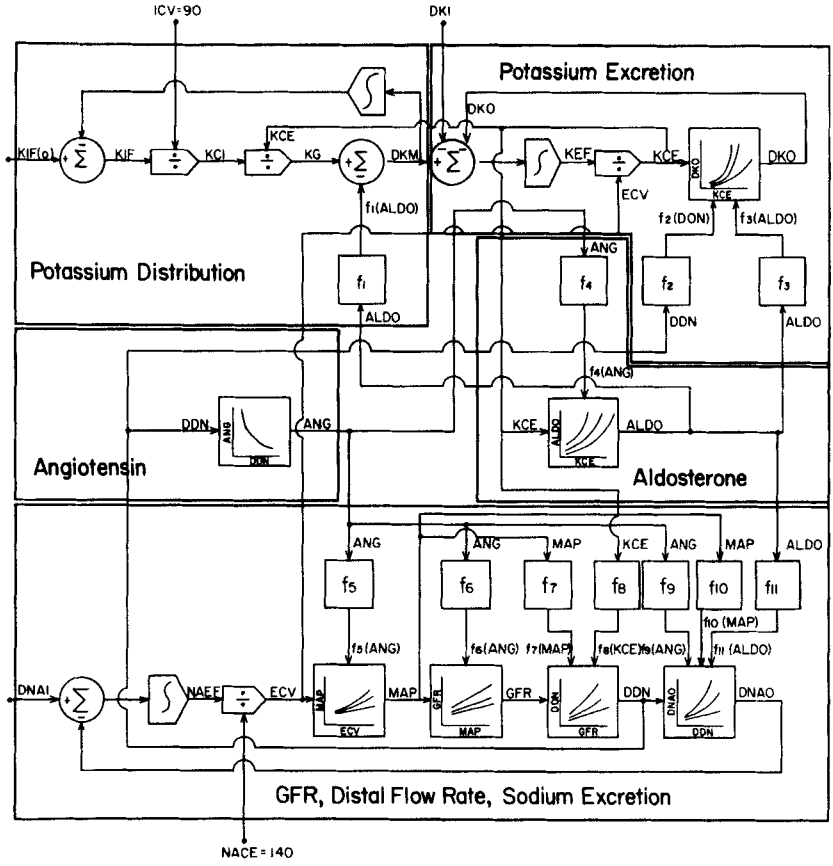

FIG. 11. Graphical representation of the hypothetical potasium control system. [From Young (1985).]

normal values for variables and parameters is given in Table IV. The functions are scaled to the proportions of the laboratory animal most frequently used in studies in this laboratory, the 22- to 25-kg mongrel dog. The basic unit of time is 1 hr. The normal Na and K intakes are both 30 mEq/day, or in the model, 1.25 mEq/hr. The model is divided into parts roughly corresponding to the tenets.

Potassium distribution is calculated in the first section. Across cell membranes in general there is assumed to be a K gradient so that normally the inside to outside concentration ratio is 140/4.2, or 33.33. Potassium concentrations are calculated from the amount of K and the volume of the extracellular fluid, which is a variable, and the intracellular volume, which is fixed. Aldosterone affects the gradient so that as aldosterone increases, the gradient also increases. If the calculated gradient is different from 33.33, K is transferred until the gradient is

TABLE II

MODEL EQUATIONS

```
1.00    POTASSIUM DISTRIBUTION

1.01    KG=KCI/KCE
1.02    AGM=(ALDO2**1.5)+32.333
1.03    DKM1=KG-AGM
1.04    DKM2=R2*(DKM1-DKM2)*ZDT+DKM2
1.05    KEF=KEF+(DKI-DK02+DKM2)*ZDT
1.06    KCE=KEF/ECV
1.07    KCI=KIF/ICV
1.08    IF(A3.GT.0)KCE=A3
1.09    KIF=KIF-DKM2*ZDT
1.10    TBK=KIF+KEF


2.00    POTASSIUM EXCRETION

2.01    D1=KCE-3.0
2.02    IF(D1.LT.0.01)D1=.01
2.03    D2=(D1**3.)*((ALDO2*.5)+.5)-.5
2.04    IF(KCE.GE.4.2)DK01=D2
2.05    D3=((KCE*.8)-2.13)*((ALDO2*.5)+.5)
2.06    IF(KCE.LT.4.2)DK01=D3
2.07    IF(DK01.LE.0.1)DK01=.1
2.08    KODDN=(DDN/.75)**3.
2.09    DK02=DK01*KODDN
2.10    DK03=R1*(DK02-DK03)*ZDT+DK03
2.11    IF(A4.GT.0.)DK03=A4


3.00    ALDOSTERONE

3.01    ALDO1=((KCE/4.2)**3.)*ANG
3.02    IF(KCE.LT.4.2)ALDO1=((KCE*.8)-2.36)*ANG
3.03    IF(ALDO1.LT.0.1)ALDO1=.1
3.04    ALDO2=R3*(ALDO1-ALDO2)*ZDT+ALDO2
3.05    IF(A1.GT.0.)ALDO2=A1


4.00    ANGIOTENSIN

4.01    ANGO=(.75/DDN)**3.
4.02    IF(ANG.LT..01)ANG=.01
4.03    ANG=R4*(ANGO-ANG)*ZDT+ANG
4.04    IF(A2.GT.0.)ANG=A2


5.00    ARTERIAL PRESSURE, GFR, DISTAL FLOW RATE, NA EXCRETION

5.01    ECV=NAEF/140.
5.02    MAP=100.*(ANG+2.)/3.*(ECV/5.)**3.
5.03    IF(A5.GT.0.)MAP=A5
5.04    NAEF=NAEF+(DNAI-DNAO)*ZDT
5.05    GFR=5.*(.95+.05*ANG)*(.5+.005*MAP)
5.06    DDN=.15*GFR*(KCE/4.2)**2.*(MAP/100.)
5.07    DDNNA=50.*DDN
5.08    DNAO=.037*DDNNA*(1./ALDO2)*(1./ANG**2.)*(MAP/100.)**4.
     400 CONTINUE
```

reestablished at the normal point. The rate of transfer is determined by a time constant of 0.02 hr, which gives a half-time response of ~50 sec. These processes are carried out by Eqs. 1.01–1.10. The equations were scaled so that the calculated values would agree with the quantitative data described earlier concerning the effect of aldosterone on K distribution (Fig. 1).

TABLE III
GLOSSARY

A1	Dummy variable used to set aldosterone concentration at specified value
A2	Dummy variable used to set angiotensin II concentration at specified level
A3	Dummy variable used to set plasma K concentration at specified level
A4	Dummy variable used to set K excretion at specified level
A5	Dummy variable used to set arterial pressure at specified level
AGM	Variable computed from aldosterone concentration used to determine rate of movement of potassium between intra- and extracellular compartments
ALDO 1	Aldosterone concentration
ALDO 2	ALDO 1 with time delay component. ALDO 2 is variable used to affect other functions; used to compute potassium excretion
ANG	Angiotensin II concentration
ANGO	Angiotensin concentration without time delay component
D1	Dummy variable used in later equations
D2	Variable which is equal to K excretion if plasma K is greater than 4.2 mEq/liter
D3	Variable which is equal to K excretion if plasma K is less than 4.2 mEq/liter
DDN	Rate of distal nephron flow
DDNNA	Rate of distal nephron sodium flow
DK1	Rate of potassium intake
DKM1	Rate of potassium movement between intra- and extracellular spaces
DKM2	DKM1 with time delay component
DKO1	Rate of potassium excretion
DKO2	Rate of potassium excretion modified by effect of distal nephron flow rate
DKO3	DKO2 with time delay
DNAI	Rate of sodium intake
DNAO	Rate of sodium excretion
ECV	Extracellular fluid volume
GFR	Glomerular filtration rate
ICV	Intracellular fluid volume
KCE	Extracellular potassium concentration; centration at specified value.
KCI	Intracellular potassium concentration
KEF	Quantity of potassium in the extracellular fluid
KG	Ratio of intra- to extracellular potassium concentration
KIF	Quantity of potassium in the intracellular fluid
KODDN	Variable computed from rate of distal nephron flow used to compute potassium excretion
MAP	Mean arterial pressure
NACE	Extracellular sodium concentration
NAEF	Quantity of sodium in the extracellular fluid
R1-4	Rate constants used to create time delays
TBK	Total body potassium quantity

TABLE IV
INITIAL CONDITIONS OF VARIABLES AND VALUES OF PARAMETERS

Variables	Paramaters
ALDO 1 = 1.0 (normalized)	A1 through A5 = 0
ALDO 2 = 1.0 (normalized)	DKI = 1.25 mEq/hr
AGM = 1.0 (normalized)	DNAI = 1.25 mEq/hr
ANG = 1.0 (normalized)	ICV = 9 liters
ANGO = 1.0 (normalized)	NACE = 140 mEq/liter
DDN = 0.75 1/hr	R1 = 0.04 hr
DKO1 = 1.25 mEq/hr	R2 = 0.02 hr
DKO2 = 1.25 mEq/hr	R3 = 1.45 hr
DKO3 = 1.25 mEq/hr	R4 = 0.12 hr
ECV = 5.0 1	
DNAO = 1.25 mEq/hr	
GFR = 5.0 1/hr	
KCE = 4.2 mEq/liter	
KCI = 140 mEQ/liter	
KEF = 21 mEq	
KIF = 1260 mEq	
KODDN = 1.0 (normalized)	
MAP = 100 mm Hg	
NAEF = 700 mEq	

Potassium excretion is calculated in Section 2.00. Above the normal level of plasma K, 4.2 mEq/liter, K excretion increased in proportion to the third power of normalized plasma K and in proportion to a linear function of normalized aldosterone concentration. Below the normal level, K excretion is linearly related to both plasma K and aldosterone concentration. Model Eqs. 2.01–2.07 (Table II) were designed so that the interaction between plasma K and aldosterone in affecting K excretion would be similar to that observed in the quantative analysis described above.

Potassium excretion is also affected by the rate of distal nephron flow; in the model, excretion is proportional to the third power of the normalized flow rate (Eqs. 2.08 and 2.09, Table II). Distal nephron flow rate is calculated from GFR, plasma K, and arterial pressure (Eq. 5.06, Table II) which, in turn, are affected directly and indirectly by the state of Na balance (Section 5.00). The equations were scaled so that the calculated effects of changing Na intake would be compatible with the data obtained in the previously described analysis of the effects of Na intake or K excretion. The time constant used to calculate changes is K excretion is 0.04 hr, which gives a half-time of response of ~100 sec (Eq. 2.10, Table II).

Aldosterone concentration is calculated in Section 3.00. Above the normal level of plasma K, 4.2 mEq/liter, aldosterone concentration is modeled as being

proportional to the third power of normalized angiotensin concentration (Eq. 3.01, Table II). Below plasma K of 4.2 mEq/liter, aldosterone is linearly proportional to plasma K and angiotensin concentrations (Eq. 3.02, Table II). These equations provide good agreement with the data obtained in the study described above concerning the interaction between plasma K and angiotensin II in stimulating aldosterone release (Fig. 10) (Young *et al.*, 1984b).

Angiotensin is calculated to be related to the third power of distal nephron flow rate (Eq. 4.01, Table II). This expression is derived from the macula densa hypothesis concerning control of renin release. The time constant for changes in angiotensin concentration is 0.12, which gives a half-time of 5 min.

In Section 5.00, extracellular volume is calculated as being equal to the quantity of Na in the extracellular space divided by 140 (Eq. 5.01, Table II). Implied in this equation is an assumption that extracellular Na concentration will be a constant 140 mEq/liter. Under normal conditions, the ADH–thirst feedback control mechanism activity regulates extracellular osmolarity and hence Na concentration to within 3% of the set point (Young *et al.*, 1977), which is ~140 mEq/liter for the dog. Mean arterial passure is calculated as being proportional to a linear function of angiotensin II concentration and a third power of normalized extracellular fluid volume (Eq. 5.02, Table II). The normal level of arterial pressure is 100 mm Hg. GFR is obtained as a function of arterial pressure and angiotensin concentration (Eq. 5.05, Table II). Distal nephron flow rate is obtained from GFR, arterial pressure, and normalized plasma K concentration (Eq. 5.06, Table II). Elevations in arterial pressure decrease proximal tubular reabsorption by altering the physical forces in the peritubular space, and elevations in extracellular K concentration inhibit reabsorption proximal to the macula densa. The rate of Na excretion is determined from the distal nephron flow rate and is inversely affected by aldosterone concentration, the square of the inverse of angiotensin concentration, and by the fourth power of normalized arterial pressure (Eqs. 5.07 and 5.08, Table II). These equations are derived from data demonstrating the powerful antinatriuretic effects of angiotensin II concentration (Hall *et al.*, 1977, 1980; Young *et al.*, 1982) and the powerful natriuretic effect of small increases in arterial pressure (Navar *et al.*, 1971; Tobian *et al.*, 1974).

IV. COMPARISON OF MODEL PREDICTIONS WITH EXPERIMENTAL OBSERVATIONS

The series of equations described above can be solved iteratively using a digital computer in order to stimulate the operation of the hypothetical control system in response to a variety of situations. By comparing the operation of the model system with experimental or clinical data, the validity of the hypothesis can be assessed. Such comparisons have been made with experimental results

obtained in this laboratory and others. Several examples chosen to emphasize the operation of portions of the model closely associated with single tenets are described below.

The effect of changes in aldosterone on potassium distribution was studied in an experiment in this laboratory in which aldosterone was infused into dogs at five times the normal rate, while plasma K and total exchangeable K were measured (Young and Jackson, 1982). The increase in aldosterone resulted in an 8% decrease in exchangeable K and a 20% decrease in plasma K over a 6-day period. The model would predict that total K would decrease 2% and plasma K would decrease 25%. Both the model prediction and the experimental observation are consistent with the proposed effect of aldosterone to shift K across cell membranes, higher levels of aldosterone moving a greater proportion of the total K into the cell interior.

The effects on potassium excretion of changes in Na and K intake and aldosterone replacement in the model and in adrenalectomized dogs have been discussed in the preceding section. It is also useful to compare the model response to changes in Na and K intake in the "intact condition" with the responses of normal dogs to the same variations in intake. The model predicts that increasing K intake from normal, 30 mEq/day, to 200 mEq/day would increase plasma K from 4.20 to 4.85 mEq/liter; whereas an increase from 4.12 to 4.65 mEq/liter was observed experimentally (Young *et al.*, 1976a). In the model, extracellular fluid volume fell by 2%, while in the experiment there was a 3% decrease due to a brief period of natriuresis following the start of the increase in K intake. Arterial pressure fell from 100 to 88 mm Hg in the simulation, from 103 to 96 mm Hg in the experiment. In the same study, Na intake was increased from 10 mEq/day to 200 mEq/day. Plasma K was unchanged in the animals but went from 4.22 to 4.04 in the simulation. In the dogs, extracellular volume increased 2% and arterial pressure did not change measurably. In the model, extracellular volume increased 15% and arterial pressure increased 24%.

The role of the renin–angiotensin system in potassium control can be observed in a study conducted by Hall *et al.* (1980). Na intake was increased from 5 to 75 to 200 to 500 mEq/day in dogs in normal conditions and while they received a continuous infusion of angiotensin II at a rate of 5.0 ng/kg/min. This infusion blocked the normal feedback regulation of angiotensin concentration. This experiment was also simulated with the model. The changes in Na intake in the normal dogs caused plasma K to vary by only a small degree; plasma K at the lowest Na intake was ~4.6 mEq/liter, and at the highest it was ~4.5 mEq/liter. The predicted values for the intact condition are 4.36 and 4.24 mEq/liter over this range of Na intake. With continuous angiotensin infusion the observed values of plasma K went from ~4.7 to 3.7 to 3.4 to 2.8 mEq/liter as Na intake was raised through the four ascending levels. The predicted values with fixed angiotensin II are 4.28 to 3.51 to 3.32 to 3.23 mEq/liter. The significant effects

of interrupting the normal feedback regulation of angiotensin II on K regulation emphasize the importance of the renin–angiotensin system acting to signal changes in distal nephron flow rate to the adrenal gland. When that signal does not respond normally during changes in Na intake, K regulation is severely impaired due to the unopposed kaliuretic effect of the increase in distal nephron flow associated with elevated Na intake.

The effects of aldosterone on K regulation were studied in an experiment in which dogs were adrenalectomized and maintained on normal Na and K intakes while aldosterone was infused at four rates, 0.33, 1.0, 2.0, and 4.5 times normal (Young and Guyton, 1977). As a result of changing the aldosterone levels for 7–10 days at a time the plasma K concentration decreased from 4.76 to 4.04 to 3.70 to 2.92 mEq/liter. The simulated experiment produced changes from 4.47 to 4.20 to 3.73 to 3.22 mEq/liter at the same four levels of aldosterone. In the model and apparently in the dogs, increasing the aldosterone concentration had a kaliuretic effect which caused progressive K depletion and a fall in plasma K. When the antikaliuretic effect of the hypokalemia matched the kaliuretic effect of the aldosterone level, K balance was achieved.

The effects of Na intake on K regulation were analyzed in an experiment partially described above. Na intake was increased from 10 to 200 mEq/day in normal dogs and in adrenalectomized dogs maintained on 2.0 times the normal level of aldosterone replacement (Young *et al.*, 1976a). In the normal dogs, the increase in Na intake did not produce a change in mean plasma K, although aldosterone concentration decreased from 1.34 normal to 0.80 normal and renin activity decreased from 1.40 to 0.80 normal. In the simulation, plasma K fell from 4.22 to 4.04 mEq/liter in the intact condition, and aldosterone went from 1.34 normal to 0.62 normal while angiotensin decreased from 1.31 to 0.72 normal. With aldosterone levels fixed at 2.0 normal, the experimental animals experienced a decrease in plasma K from 3.71 to 2.61 mEq/liter when Na intake was increased, and in the simulation plasma K fell from 3.99 to 3.36 mEq/liter. In this case, the natriuretic effect of the increase in distal nephron flow rate resulting from the increase in Na intake was offset in the normal condition by a decrease in aldosterone concentration signaled by a fall in angiotensin concentration. When aldosterone concentration was fixed, the increase in Na intake and distal nephron flow had a natriuretic effect that could not be balanced by a decrease in aldosterone concentration. Therefore, a negative K balance proceeded until the decrease in plasma K had an antikaliuretic effect of sufficient magnitude to balance the kaliuretic effect of the elevated distal nephron flow rate.

To the present time, the operation of the model has been in agreement with results of all of the many experimental manipulations with which it has been compared. While the agreement between model predictions and data does not represent an exclusive proof of the validity of the model, the consistent sim-

ilarities between data and predictions in response to a very wide range of challenges provide support for the hypotheses inherent in the model. With each subsequent case of agreement between simulation and experimental observation, there is an increased probability that the hypothetical control system closely corresponds to the actual long-term potassium control system.

V. QUESTIONS RAISED BY THE MODEL

Constructing the model raised several important questions that can only be answered by direct experimental investigation. Several of these are discussed below.

Do changes in plasma K affect renal blood flow and GFR autoregulation? The author could not construct a reasonable model of K regulation without including an effect of plasma K on proximal tubular reabsorption. If the effect is significant, then changes in plasma K may affect the feedback regulation of GFR and renal blood flow.

Does aldosterone affect the transmembrane K concentration gradient across cell membranes throughout the body? A positive answer to this question is suggested by the agreement between simulated K distribution and experimental data. However, this fundamental question demands an extensive investigation.

What mechanisms are responsible for the very powerful effect of changes in plasma K on K excretion? The dramatic change in excretion caused by changes in plasma K of a few percentages suggests that several powerful mechanisms acting in series are affected by K concentration. Stanton and Giebisch recently found that much of the effect occurs in the distal tubule (1983). The mechanisms of the distal effect and the possibility of other sites of action remain to be studied.

Can changes in K intake affect blood pressure over a period of days or weeks? The model predicts that a high K intake will result in a negative Na balance and a decrease in angiotensin levels which combine to produce a fall in blood pressure. This effect has been difficult to reproduce experimentally or clinically, although some studies have shown a positive effect (Young *et al.*, 1976b; Suzuki *et al.*, 1981). This is an intriguing and clinically relevant area of study.

Can long-term alterations in K intake affect blood pressure over a lifetime? The model predicts that a low K intake will result in an elevated GFR due to the positive effect of subnormal plasma K on proximal tubular Na reabsorption. Brenner *et al.* have recently postulated that long-term elevations in filtration pressure and filtration rate per glomerulus resulting from a high protein intake or from reduced renal mass cause glomerular damage that leads to sustained hypertension (Brenner *et al.*, 1982; Brenner, 1983). Can increases in GFR resulting from a lifetime of a relatively low K–high Na diet produce a situation such as that proposed by Brenner which could result in hypertension? Intriguingly, a

high K–low Na diet is consumed by primitive tribes in whom hypertension is unknown, and when members of such groups change to a modern diet high in Na and low in K, the incidence of hypertension rises dramatically (Allen *et al.*, 1982). In addition, Dahl *et al.* have found that a high K intake protects certain strains of rat from hypertension produced by a lifetime of high Na intake (Dahl *et al.*, 1972), and recently, Tobian *et al.* reported a protection by a high K intake of the glomerulus from lesions produced by a high salt intake in rats (Tobian *et al.*, 1984). The possibility that K intake may affect lifetime blood pressure and population blood pressure is of considerable importance and must be thoroughly investigated.

VI. CLINICALLY RELEVANT MODEL PREDICTIONS

Although the model was designed to test the validity of a set of hypotheses concerning K regulation, results of some of the simulations may be of clinical relevance, assuming the model is an accurate representation of the long-term control system. Several examples are discussed below. In general, these predictions are untested and must be viewed as hypothetical until they are thoroughly studied.

Drugs that affect the activity of the renin–angiotensin system, whether they be release inhibitors, such as propranolol, or renin or converting enzyme inhibitors, will affect K regulation only in states of Na depletion, according to the model. Therefore, in patients consuming a normal Na intake, such drugs would not have important effects on plasma K, although in patients with a low Na intake or taking diuretics that do not directly induce a kaliuresis, inhibition of the renin–angiotensin system would be expected to cause hyperkalemia.

Aldosterone administration may be useful in lowering plasma K in cases of acute, potentially lethal hyperkalemia. While latent time prior to the onset of action may be longer for aldosterone than for the more commonly employed measures such as insulin, the duration of action would also be much longer. Aldosterone would act first by inducing an inward shift of K into cells and second by increasing K excretion. Large amounts of aldosterone could be given without fear of serious side effects.

Patients with inadequate or uncontrolled aldosterone secretion may be maintained in proper Na and K balance by adjusting the ratio of Na and K intake rather than the absolute amount of either. Addisonian patients may not need any mineralocorticoid replacement if Na and K are consumed in the appropriate ratio, and Cushing's syndrome patients may avoid hypokalemia if Na intake is reduced. These predictions are derived from the concept that aldosterone is not the principal regulator of either Na or K excretion, but instead the hormone allows the kidney to excrete simultaneously a wide range of Na and K intake. Angioten-

sin is a more powerful and active regulator of Na excretion than is aldosterone, and the level of plasma K has a much greater effect on K excretion than does aldosterone. However, if aldosterone levels are inappropriate, then K inbalance will occur unless a given ratio of Na and K is consumed. If plasma K is lower than desired in a patient with hyperaldosteronism, for example, it may be increased by decreasing Na intake and/or increasing K intake, either of which would decrease the Na/K ratio. Once the appropriate ratio is determined, approximate K balance could be maintained regardless of the absolute levels of Na or K intake as long as the ratio was maintained constant and aldosterone levels did not change.

One could predict from the operation of the model that a low K intake could increase GFR. This would result from the effect of hypokalemia to increase proximal reabsorption, thereby tending to decrease flow to the macula densa sensor that is involved in GFR autoregulation. The sensor would signal GFR to increase until flow at the sensor returned to the set point. Therefore, theoretically a low K intake could increase GFR, possibly to the benefit of the patient with renal insufficiency.

VII. CONCLUSION

Potassium is regulated over periods of days and weeks by a system whose components affect potassium secretion by the cells of the distal nephron and the distribution of potassium across cell membranes throughout the body. Because of the complexity of the system, its function could not be analyzed by traditional direct experimental techniques. Instead, a hypothetical system was proposed and eventually cast as a mathematical model. Data were obtained from experiments designed to quantitatively assess the functions of the components and were used to formulate the model equations. The principal concepts upon which the model is based include the following: (1) Aldosterone regulates the distribution of K between the intra- and extracellular spaces, higher aldosterone concentrations shifting a greater proportion of K into the cells. (2) K excretion is most powerfully affected by the plasma K concentration, excretion increasing in proportion to the third power of a function of plasma K above the normal level. Aldosterone concentration and distal nephron flow both affect K excretion, but not as strongly as changes in plasma K. (3) Aldosterone is regulated by a multiplicative interaction between angiotensin II and plasma K concentration. (4) Renin release is regulated by the rate of flow into the distal nephron. (5) Distal nephron flow rate is a function of GFR which is modified by the level of plasma K as well as by arterial pressure. Increasing plasma K decreases proximal tubular reabsorption, thereby increasing distal flow rate. General agreement between model solutions and experimental results have been found which supports the validity of the

hypothesis as represented by the model. With each case of agreement between the model solutions and experimental findings, the probability increases that the hypothetical control system closely resembles the actual system.

ACKNOWLEDGMENT

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Part III

Renal and Extrarenal Control of Potassium: Diuretics and Pathophysiology

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Chapter 13

Regulation of Extrarenal Potassium Homeostasis by Insulin and Catecholamines

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I. INTRODUCTION

The maintenance of normal potassium homeostasis is dependent upon both renal and extrarenal mechanisms (Wright and Giebisch, 1985; DeFronzo and Bia, 1985). Long-term potassium balance is primarily regulated by the kidney (Wright and Giebisch, 1985). In contrast, the extrarenal tissues play a major role in the regulation of acute potassium metabolism (DeFronzo and Bia, 1985). In the present review, we shall focus on the hormonal control of extrarenal potassium homeostasis, using insulin and catecholamines as model systems.

II. POTASSIUM DISTRIBUTION

In healthy subjects, total body potassium stores amount to about 50 mEq/kg body weight or 3500 mEq for a 70-kg man (DeFronzo and Bia, 1985). As can be seen in Fig. 1, the great majority of this potassium resides within cells, primarily muscle. Only 2% of total body potassium, or 65 mEq, circulates in the extracellular compartment at concentrations ranging from 3.5 to 5.0 mEq/liter. In contrast, the intracellular potassium concentration is quite high, 140–150 mEq/liter. The intracellular to extracellular ratio of potassium is therefore quite high (40–50), and the maintenance of this high concentration gradient is critical

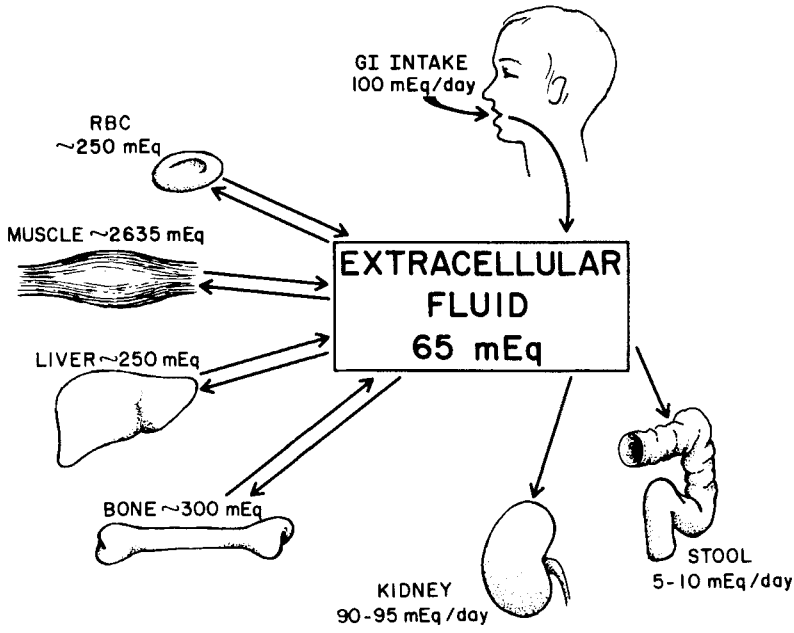

FIG. 1. Schematic representation of total body potassium distribution and the maintenance of potassium homeostasis in healthy subjects. See text for a detailed discussion.

for normal cell function, since it is the primary determinant of the resting membrane potential. If as little as 1% of the total body potassium stores is lost from (or added to) the extracellular compartment, the plasma potassium concentration will decrease (or increase) by 50%. This would markedly alter the intracellular to extracellular potassium concentration ratio and have a catastrophic effect on cardiac and neuromuscular function. Likewise, redistribution of a similar amount of potassium from intracellular to extracellular compartment without any change in total body potassium would have similar adverse effects on cell function. It is important, therefore, that both the total amount of potassium within the body as well as the distribution of potassium between intracellular/extracellular compartments be tightly regulated.

Maintenance of total body potassium stores, i.e., *chronic* potassium balance, is the primary domain of the kidney. Each day, the average person ingests about 100, mEq of potassium and, of this, ~90 mEq is excreted via the kidney and 10 mEq by the gastrointestinal tract. In contrast, *acute* potassium homeostasis is regulated, in large part, by the extrarenal tissues. In man and animals, during the

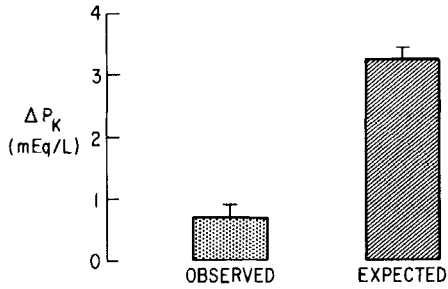

FIG. 2. Extrarenal distribution of an acutely administered intravenous potassium load (0.75 mEq/kg) in healthy subjects. During the first 4–6 hr following administration of KCl, ~40% was excreted. If all of the retained potassium remained in the extracellular fluid compartment, marked hyperkalemia (EXPECTED) would have ensued. This did not occur (OBSERVED), since over 70–80% of the retained potassium was translocated into cells.

4- to 6-hr period immediately following potassium ingestion or infusion, less than half of the administered potassium load is excreted in the urine (DeFronzo *et al.*, 1978, 1979a, 1980b; Gonick *et al.*, 1971) (Fig. 2). If all of the retained potassium remained in the extracellular fluid compartment, life-threatening hyperkalemia would result. Fortunately, this does not occur, since over 70–80% of the administered potassium load is translocated into cells, primarily muscle and liver. A number of regulatory factors, both hormonal and nonhormonal, play an important role in maintaining the normal distribution of potassium between the intracellular and extracellular environment (DeFronzo and Bia, 1978) (Table I). In the present review, we shall focus on two hormones, insulin and catecholamines, whose contribution to extrarenal potassium metabolism have been well worked out.

TABLE I
FACTORS KNOWN TO BE RESPONSIBLE FOR THE NORMAL REGULATION OF EXTRARENAL POTASSIUM HOMEOSTASIS

Hormonal
Insulin
Catecholamines
Glucagon
Mineralocorticoids
Glucocorticoids
Nonhormonal
Acid–base balance
Tonicity

III. INSULIN

The important role of insulin in the disposition of an exogenously (DeFronzo *et al.*, 1978 1980b; Davidson and Hiatt, 1972; Dluhy *et al.*, 1972; Hiatt *et al.*, 1972, 1973, 1974, 1976; Pettit and Vick, 1974b; Pettit *et al.*, 1975; Santeusano *et al.*, 1973) administered potassium load is well established. Following KCl infusion in dogs to elevate the plasma potassium concentration by 1–1.5 mEq/liter or greater (Davidson and Hiatt, 1972; Dluhy *et al.*, 1972; Hiatt *et al.*, 1972, 1973, 1974, 1976; Pettit and Vick, 1974b; Pettit *et al.*, 1975; Santeusano *et al.*, 1973), peripheral immunoreactive insulin levels increase 2- to 3-fold. A similar stimulatory effect of potassium on insulin secretion has been observed *in vitro*, indicating a direct effect of the potassium ion on insulin secretion by the β cell (Gomez and Curvy, 1973; Grodsky and Bennett, 1966; Grodsky *et al.*, 1967; Hales and Milner, 1968; Henquin and Lambert, 1974; Howell and Taylor, 1968). Pancreatectomy leads to a marked deterioration in potassium tolerance and lethal cardiac toxicity (Hiatt *et al.*, 1973, 1974, 1976; Pettit and Vick, 1974b; Santeusano *et al.*, 1973). If pancreatectomized animals are replaced with exogenous insulin, potassium tolerance is returned to normal (Hiatt *et al.*, 1973; Santeusano *et al.*, 1973).

The studies cited above indicate that large increases (>1–1.5 mEq/liter) in plasma potassium stimulate insulin secretion and that the resultant hyperinsulinemia, in turn, enhances the disposal of an acute potassium load. However, with small, more physiological increments in the plasma potassium concentration (0.3–0.7 mEq/liter), circulating insulin levels remain unchanged (DeFronzo *et al.*, 1978, 1979a, 1980b, 1981; Dluhy *et al.*; 1972; Rosa *et al.*, 1980; Sterns *et al.*, 1979). To examine whether insulin contributes to the regulation of potassium metabolism under these more physiological conditions of hyperkalemia, DeFronzo *et al.* (1978) infused KCl into awake dogs to raise the plasma potassium concentration by 0.4–0.6 mEq/liter (Fig. 3). Under these conditions, peripheral insulin levels remained unchanged. When somatostatin was infused with KCl, circulating insulin levels fell by 50% and the rise in plasma potassium concentration was 2.5-fold greater than when the basal insulin concentration was maintained. When insulin was infused with somatostatin to keep basal insulin levels constant, potassium tolerance was restored to normal. Urinary potassium excretion was similar in all three studies, indicating that the deterioration in potassium tolerance following somatostatin-induced insulinopenia was entirely explained by decreased cellular uptake of potassium. These results emphasize the importance of basal insulin levels in the regulation of acute potassium homeostatis. To further examine the role of basal insulin secretion in potassium homeostasis, somatostatin was infused into normal and diabetic man (DeFronzo *et al.*, 1978). In nondiabetic subjects, a predictable rise in plasma potassium of 0.5 mEq/liter ensued, and this could be reversed by an exogenous insulin infusion designed to

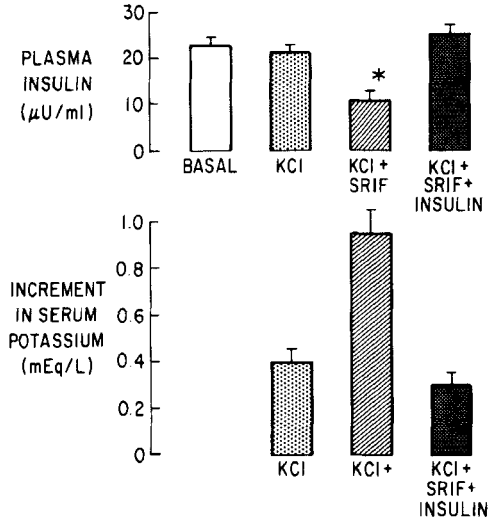

FIG. 3. The effect of potassium chloride (KCl) infusion alone, KCl plus somatostatin (SRIF) infusion, and KCl plus SRIF plus insulin on plasma potassium concentration in dogs.

maintain basal insulin levels constant (Fig. 4). Similar results have been presented in man (Massara *et al.*, 1980) and dogs (Pettit and Vick, 1974b). In non-insulin-dependent diabetic patients who have normal or elevated fasting insulin levels, inhibition of basal insulin secretion with somatostatin resulted in a similar hyperkalemic response. In insulin-dependent diabetics who have a complete lack of insulin, somatostatin had no effect on the plasma potassium concentration (Fig. 4). These results indicate that a normal basal rate of insulin secretion is a

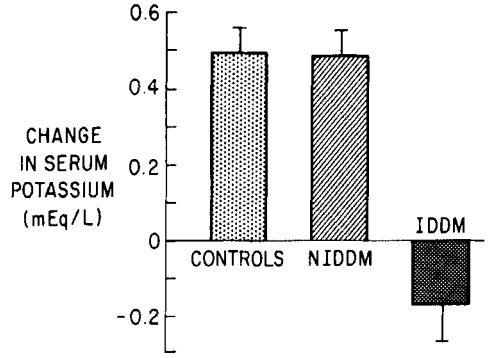

FIG. 4. The effect of somatostatin infusion on the plasma potassium concentration in normal controls, maturity onset (Type II) diabetics, and juvenile onset (Type I) diabetics.

necessary requirement for the maintenance of normokalemia in the postabsorptive state. Since urinary potassium excretion was unchanged following somatostatin and since similar results have been reported in nephrectomized and ureteral ligated dogs, it is clear that the effect of insulin is independent of the kidneys and involves extrarenal tissues (Hiatt *et al.*, 1973, 1974, 1976; Pettit *et al.*, 1975). Further, clearance and micropuncture studies have shown that insulin has no direct effect on renal potassium excretion (DeFronzo *et al.*, 1975, 1976). The importance of this relationship between basal insulin secretion and potassium homeostasis is underscored by the frequent occurrence of hyperkalemia in insulin-dependent diabetic patients in the absence of uremia or acidosis (DeFronzo, 1980).

A number of techniques have been employed to define those extrarenal tissues involved in the insulin-mediated stimulation of potassium uptake. Using the forearm perfusion technique, Zierler and Rabinowitz (1964) and Andres *et al.* (1962) demonstrated that both deep (muscle) and superficial (adipose) forearm tissue in man are responsible for the increase in potassium uptake following insulin infusion. When insulin was infused into the brachial artery to increase the plasma insulin concentration perfusing the forearm by as little as 40 μU/ml, potassium uptake by both deep and superficial tissues was markedly stimulated, even though glucose uptake remained unchanged. In addition to these *in vivo* results, a number of *in vitro* studies have documented that insulin causes a net uptake of potasisum by both skeletal (Clausen and Hansen, 1977; Crese and Northover, 1961; Gourley, 1961, 1965; Gourley and Bethea, 1964; Zierler, 1957, 1959, 1968; Zierler *et al.*, 1966) and cardiac (Regan *et al.*, 1963; Rogers *et al.*, 1977a) muscle, as well as by adipose tissues (Clausen and Hansen, 1977; Gourley and Bethea, 1964; Clausen *et al.*, 1969; Perry and Hales, 1969), and that this action of insulin is independent of its effect on glucose metabolism (Zierler, 1951, 1968).

In addition to muscle and adipose tissue, the liver also has been shown to play an important role in potassium disposal following hyperinsulinemia. Fenn (1939) was the first to show that carbohydrate feeding, with its resultant stimulation of insulin secretion, enhanced hepatic potassium uptake. A direct effect of insulin on hepatic potassium uptake was subsequently documented using the isolated perfused dog (Kestens *et al.*, 1963) and rat (Burton *et al.*, 1967; Mortimore, 1961) liver. Furthermore, this stimulatory effect of insulin on hepatic potassium uptake was shown to be independent of glucose uptake. Using the insulin clamp technique in combination with hepatic vein catheterization, DeFronzo *et al.* (1980a) have quantitated the effect of hyperinsulinemia on the hepatic disposal of potassium in man (Fig. 5). When the plasma insulin concentration was raised by 25–1000 μU/ml, while maintaining euglycemia (DeFronzo *et al.*, 1979b), a dose-related decline in plasma potassium concentration was observed (Fig. 5, top). A rise in plasma insulin concentration of as little as 25 μU/ml resulted in a

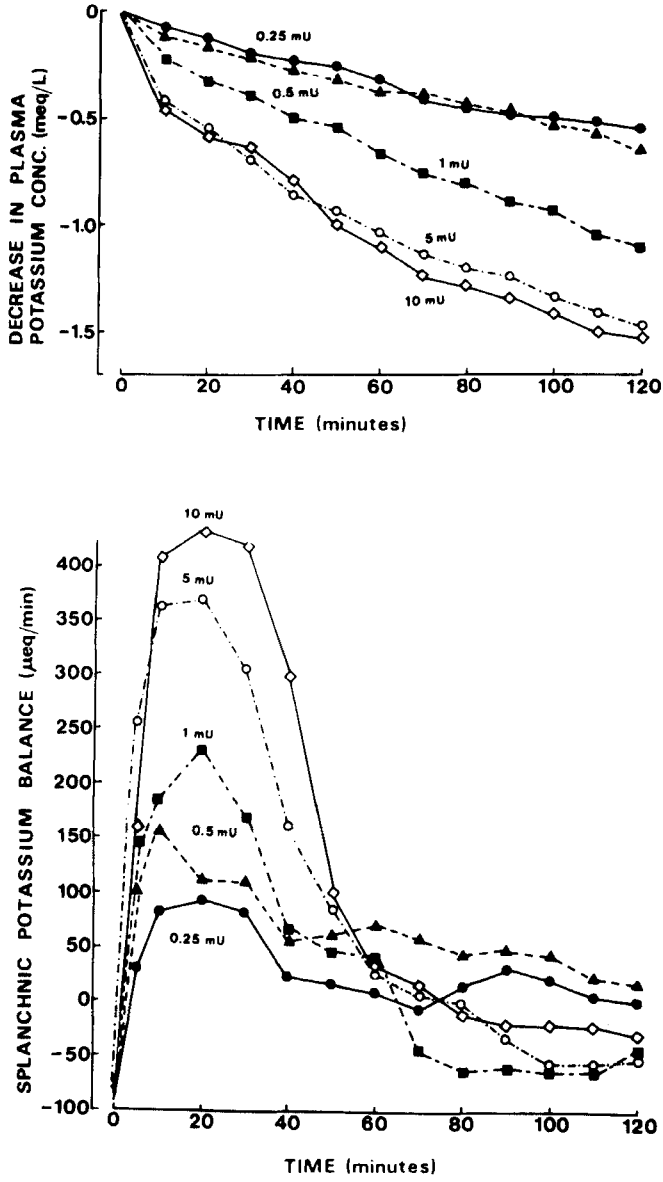

FIG. 5. Top: Time-related decrease in plasma potassium concentration during euglycemic hyperinsulinemia. Insulin was infused at 0.25, 0.5, 1.0, and 10 mU/kg·min and resulted in plasma insulin levels of ~25, 50, 100, 500, and 1000 μU/ml above basal. Bottom: Time-related change in splanchnic potassium balance during euglycemic hyperinsulinemia.

0.58 ± 0.11 mEq/liter decline in plasma potassium after 2 hr. Splanchnic (hepatic plus gastrointestinal) uptake of potassium was stimulated by hyperinsulinemia in a dose-dependent fashion (Fig. 5, lower). During the first hour following insulin, 70% of the decline in plasma potassium could be accounted for by splanchnic uptake and 30% by peripheral tissue uptake. During the second hour, this relationship became reversed. Splanchnic uptake decreased precipitously and a net splanchnic release of potassium occurred in every subject in whom the plasma potassium concentration declined by more than 1 mEq/liter. Nonetheless, the plasma potassium concentration continued to drop. During this period peripheral tissues, primarily muscle, became the major site of potassium disposal. If hepatic potassium uptake were a saturable process, one would expect the splanchnic uptake to increase initially and then plateau. One would not, however, anticipate a net efflux or even a decline in potassium uptake. To examine the mechanism of this phenomenon, a simultaneous infusion of potassium was administered with insulin to maintain the plasma potassium concentration constant at the basal level. Under these experimental conditions, splanchnic potassium uptake continued unabated. These results indicate that the net reversal of splanchnic potassium uptake was due to the increasing concentration difference for potassium between the hepatocyte and plasma. Consistent with previous results obtained with the isolated perfused liver (Kestens *et al.*, 1963; Burton *et al.*, 1967; Mortimore, 1961) and with muscle (Zierler, 1951, 1959b, 1968; Andres *et al.*, 1962), DeFronzo *et al.* (1980a) also failed to observe any correlation between splanchnic potassium uptake and splanchnic glucose uptake.

From the preceding discussion, it is clear that insulin plays an important role in extrarenal potassium homeostasis. Small physiological increments in the plasma insulin concentration (25–40 μU/ml) augment potassium uptake by muscle, liver, adipose, and probably other tissues. An increase in the plasma potassium concentration in excess of 1.0–1.5 mEq/liter stimulates insulin secretion, and the resultant hyperinsulinemia, in turn, promotes the cellular uptake of potassium. Even in the absence of a detectable rise in peripheral insulin concentration, the simple maintenance of basal insulinemia is a necessary requirement for the disposal of an acute potassium load. However, before it can be concluded that the role of insulin is purely permissive in facilitating potassium uptake when the rise in plasma potassium concentration is small (<1.0 mEq/liter), it must be shown that portal insulin levels do not increase. The normal portal to peripheral insulin concentration gradient is about 3:1 (Blackard and Nelson, 1970). Thus, significant portal hyperinsulinemia can occur without any change in peripheral levels. Since DeFronzo *et al.* (1980a) have shown that an increase in portal insulin concentration of only 25 μU/ml can significantly augment splanchnic potassium uptake, it is possible that the role of insulin is both active (i.e., on the liver) and permissive (i.e., on muscle, since peripheral insulin levels are not

increased). Support for this thesis comes from two sources. First, Hiatt *et al.* (1972) have reported significant increases in the portal venous insulin concentration when the plasma potassium concentration is raised by only 0.5–1.0 mEq/liter. Second, we have documented an increase in plasma C peptide concentration following the infusion of KCl in doses that caused no change in peripheral insulin levels (unpublished observations). Since C peptide is released with insulin in a 1:1 molar ratio but is not extracted by the liver (Kuhl *et al.*, 1978), it provides a measure of pancreatic insulin release into the portal circulation. On the basis of these results, one can construct a positive-feedback loop, as shown in Fig. 6. Hyperkalemia stimulates insulin secretion by the pancreas. The resultant hyperinsulinemia, independent of its effect on glucose metabolism, enhances potassium uptake by the liver, muscle, and other tissues of the body, thereby returning the plasma potassium concentration to normal. The restoration of normokalemia, in turn, removes the stimulus for continued insulin secretion.

In recent years, much effort has been directed at defining the cellular mechanism(s) via which insulin enhances potassium uptake. Although much has been learned about how insulin stimulates potassium transport, significant controversy still remains. The first step in insulin action involves its binding to a specific receptor on the cell surface (Czech, 1981). Such receptors are present on all insulin target tissues. Trypsinization of cells obliterates the effect of insulin on ion transport and membrane potential, indicating that the response requires an intact cell surface receptor (Resh *et al.*, 1980) (Fig. 7). Furthermore, the ability of insulin to stimulate ^{86}Rb uptake correlates closely with insulin binding, and both insulin binding and ^{86}Rb uptake (Resh *et al.*, 1980) are blocked by trypsinization.

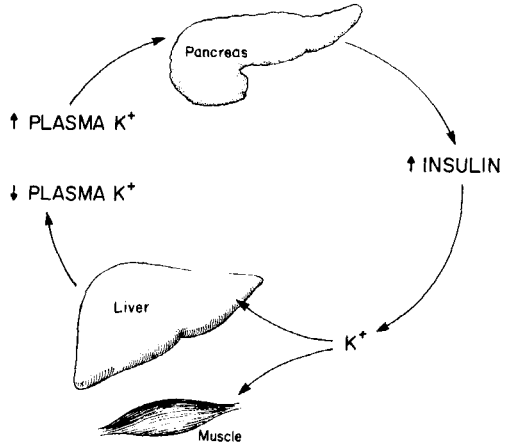

FIG. 6. Feedback loop relating changes in plasma potassium concentration to changes in insulin secretion by the pancreatic β cells.

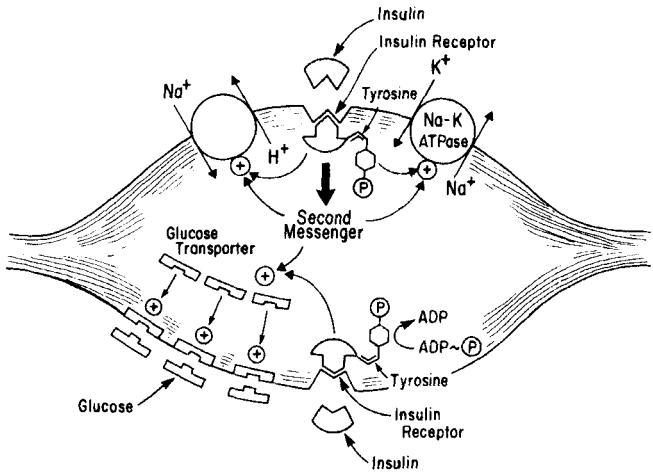

FIG. 7. Schematic representation of the cellular action of insulin on potassium and glucose transport. See text for a detailed discussion.

After insulin has bound to its receptor, the cell membrane becomes polarized. This hyperpolarization has been demonstrated in muscle (Zierler, 1951, 1957, 1959a; DeMello, 1967; LaManna and Ferrier, 1981; Moore and Robovsky, 1979) as well as a number of other tissues (DeMello, 1967; Beigelman and Hollander, 1964, 1965; Crabbe and Francois, 1967; Miller and Constant, 1960; Rehm *et al.*, 1961); it occurs rapidly, can be shown in the absence of glucose (DeMello, 1967; Moore and Rabovsky, 1978; Rehm *et al.*, 1961; Zierler, 1959b), and can be demonstrated both *in vitro* and *in vivo* (Flatman and Clausen, 1979). Zierler and Rogers (1981) have demonstrated that the addition of insulin to the incubation medium produces its hyperpolarizing effect on muscle within 1 min, and if insulin is injected into the surface with a micropipette, hyperpolarization occurs within seconds. The change in membrane potential (see reviews by Zierler, 1973; Moore, 1983) cannot be explained by the increased ratio of intracellular to extracellular potassium concentration, since the hyperpolarization precedes any detectable increase in the intracellular potassium concentration (Zierler, 1957, 1959b, 1973; Moore and Robovsky, 1979; Flatman and Clausen, 1979). Two explanations have been offered for the hyperpolarizing action of insulin: (1) either a decrease in the ratio of sodium to potassium permeability, or (2) stimulation of the Na^+–K^+ pump, resulting in the generation of an outward electric current. Evidence to support an increased K/Na permeability has been provided by Zierler *et al.* (1966) and Zierler (1973). However, it is also well established that insulin activates the Na^+–K^+ pump (Crese and Northover, 1961; Moore, 1973, 1983; Clausen and Kohn, 1977; Erlij and Grinstein, 1982), which is intimately related to, if not identical with, the enzyme Na^+,K^+-

ATPase (Hougen *et al.*, 1978; Jarrett and Smith, 1974; Sjodin, 1982) (Fig. 7). Since the Na^+–K^+ pump is electrogenic (Kernan, 1962; Keynes and Rybova, 1963; Thomas, 1972), i.e., the number of Na^+ ions extruded is greater than the number of K^+ ions pumped in, a net outward current is generated, leading to hyperpolarization of the cell membrane. The rapid time course of activation of the Na^+–K^+ pump suggests that insulin works by increasing the affinity of the pump for Na and not by increasing the number of pump sites. This thesis has been confirmed by ouabain binding studies (Clausen and Kohn, 1977; Clausen, 1986; Resh, 1982).

The ability of insulin to stimulate the net influx of potassium into cells is well documented (Gourley, 1961; Gourley and Bethea, 1964; Zierler, 1968, 1973; Moore, 1973, 1983; Clausen and Kohn, 1977; Berg and Iversen, 1976; Manery *et al.*, 1977). As discussed in the preceding paragraph, the primary action of insulin is to hyperpolarize the cell membrane. The resultant hyperpolarization is, of itself, sufficient to account for the net cellular accumulation of potassium by passive distribution along the electrical gradient created by sodium efflux. However, controversy exists as to whether the accumulation of potassium within the muscle cell primarily results from inhibition of efflux (Zierler, 1968) or stimulation of influx (Gourley, 1961, 1965; Gourley and Bethea, 1964). At present it is not clear whether this divergence of opinion is the result of different techniques (Zierler measured efflux while Gourley measured influx) or different experimental animals (rat versus frog) employed by different investigators.

Although it is well established that activation of the Na^+–K^+ pump and the enzyme Na^+,K^+-ATPase (Moore, 1983; Hougen *et al.*, 1973; Jarrett and Smith, 1974; Sjodin, 1982; Gavryck *et al.*, 1975) is intimately involved in the cellular action of insulin to stimulate potassium transport, the second messenger that mediates this effect has remained elusive. There is little evidence to support a role of cyclic AMP in this action (Moore, 1983). This is in contrast to epinephrine (see subsequent discussion), where it is clear that cyclic AMP generation is closely linked to catecholamine-mediated increase in potassium transport (Flatman and Clausen, 1979; Cheng *et al.*, 1976; Clausen and Flatman, 1977; Clausen, 1983; Rogers *et al.*, 1977b). Two observations support this contention. First, propranolol, which blocks epinephrine-stimulated potassium transport, has no effect on insulin-stimulated potassium uptake (Clausen and Martin, 1977; Minaker and Rowe, 1982). Second, the effects of epinephrine and insulin to augment potassium uptake are additive (Flatman and Clausen, 1979; Moore, 1983). It is also unlikely that the synthesis of new protein can explain the acute effects of insulin on sodium/potassium transport in muscle and other extrarenal tissues. First, the onset of insulin's action is quite rapid. Second, neither actinomycin D (an inhibitor of messenger RNA synthesis) nor puromycin/cycloheximide (inhibitors of protein synthesis) block sodium transport or the short-circuit current generated after the addition of insulin to the toad urinary bladder

(Cobb *et al.*, 1981; Crabbe, 1973; Crane, 1977; MacKnight *et al.*, 1980; Wiesman *et al.*, 1976). This observation stands in contrast to aldosterone-stimulated sodium transport, which is blocked by both actinomycin D and puromycin.

Recent studies from several laboratories have provided new and exciting information that the second messenger for insulin action may be the insulin receptor itself. Both Kasuaga and colleagues (1982) and Czech (1981) have shown that insulin binding leads to the phosphorylation of its own receptor and the subsequent activation of a cascade of phosphorylation–dephosphorylation reactions within the cell. These changes, in turn, are associated with an alteration in the lipid matrix and fluidity of the plasma membrane (Amatruda and Finch, 1973; Kimelberg, 1977; Luly and Shinitzky, 1979). The alterations in membrane structure has been postulated to be responsible for some of the many varied actions of insulin. In addition, Zierler and colleagues (1973, 1980) have provided evidence that hyperpolarization per se may account for some of the actions of insulin, including the increase in potassium influx and the stimulation of glucose transport. In fact, it has been postulated that many polypeptide hormones may exert their effects via alteration of the membrane potential difference (Moore, 1983).

Stimulation of the Na^+-K^+ pump by insulin is well established. However, it is now recognized that insulin activates a variety of other transport systems (Fig. 7). Thus, Moore and co-workers have demonstrated that insulin stimulates a plasma membrane Na:H exchange system that can be inhibited by amiloride (Fidelman *et al.*, 1982; Moore *et al.*, 1979; Moore, 1979, 1981; Moore and Gupta, 1980). This has important physiological consequences. As hydrogen ion is actively extruded from the cell, the intracellular pH rises, resulting in the stimulation of phosphofructokinase (a key enzyme in the glycolytic cycle) and enhanced glycolytic flux (Fidelman *et al.*, 1982; Moore *et al.*, 1979; Moore, 1979, 1981; Moore and Gupta, 1980). Eventually, the sodium, which has accumulated within the cell in exchange for hydrogen, must be pumped out. Although not yet examined, it is of interest to speculate that this occurs in exchange with potassium and is, in part, responsible for insulin-stimulated influx of potassium. If true, amiloride, an inhibitor of the Na–H pump, should inhibit insulin-stimulated potassium transport.

Many studies have shown that the stimulatory effects of insulin on potassium and glucose transport are clearly separable (see previous discussion). This is not surprising, since insulin stimulates hexose transport by recruiting glucose receptors from the endoplasmic reticulum and inserting them into the cell membrane (Cushman and Wardzala, 1980; Kono *et al.*, 1982), whereas the effect of insulin on potassium transport appears to be electrogenically mediated. With respect to amino acid transport, there is abundant evidence that stimulation of sodium transport and the resultant electrical potential difference are closely related to the intracellular accumulation of type A amino acids (Crane, 1979; Evers *et al.*,

1976; Johnstone, 1979; Lever, 1977; Pershedsingh *et al.*, 1978). Finally, insulin has been shown to enhance the cellular efflux of $^{45}Ca^{2+}$ in a variety of tissues (Calusen and Martin, 1977). Since changes in the cellular distribution of calcium have been proposed as a mediator of hormone action, it is possible that some of the effects of insulin are mediated via this second messenger system. How, if at all, such changes are related to alterations in potassium transport remains to be examined.

Potassium transport is now known to occur through specific potassium channels, and over the past decade considerable investigation has been devoted to defining the structure, conduction properties and selectivity of these channels in extrarenal tissues, primarily muscle and nerve. The reader is referred to a recent excellent review of the subject by Latorre and Miller (1983), and only a few brief comments will be made here. The cell membrane represents a complex lipid bilayer, and the energy cost of transporting ions through this structure is quite high. In order to circumvent this problem and conserve energy, there has evolved a class of membrane proteins whose only function is to facilitate the transport of charged species, including potassium, into and out of the cell. In a certain sense, these membrane proteins can be viewed as enzymes (Hille, 1975) in that they minimize the energy requirements for ion diffusion, display saturation kinetics, demonstrate competitive inhibition, and manifest a high degree of substrate (ionic) specificity (Latorre and Miller, 1983). Using "patch recording" methods (Methfessel and Boheim, 1982; Pallotta *et al.*, 1981) and plasma bilayer techniques (Latorre *et al.*, 1982; Miller, 1978), a variety of potassium selective channels have been observed in animal cell membranes. The chemical structure of these potassium ion channels, as well as their conducting behavior, is quite complex and beyond the scope of this chapter. A detailed description can be found in the review by Latorre and Miller (1983). At present, there is no information as to how insulin or other hormones may interact with these potassium selective channels. For instance, does insulin work by increasing the number of these channels, unmasking preexisting channels, or altering their conformational structure? Because of the very rapid onset of insulin's action, it seems most likely that the hormone acts by inducing a conformational change that would allow previously closed channels to conduct potassium. The opening and closing of these channels could be driven by changes in the membrane potential (Zierler and Rogers, 1980), phosphorylation of the membrane-bound insulin receptor (Czech, 1981; Kasuga *et al.*, 1982), or the release of specific chemical transmitters, i.e., second messengers (Clausen and Martin, 1977; Jarrett and Seals, 1979). Although the interaction between insulin and these potassium selective channels has received little attention, the development of patch recording, planar bilayer, and "macroscopic noise" techniques (Latorre and Miller, 1983) has made such investigation possible. Particularly exciting are recent reports in which the potassium channel has been isolated and inserted into phospholipid

bilayers of sarcoplasmic reticulum membrane vesicles (Labarca *et al.*, 1980; Coronado *et al.*, 1980; Miller, 1978.

IV. CLINICAL IMPLICATIONS FOR THE DIABETIC

From the preceding discussion, it is obvious that the diabetic individual is at marked risk to develop clinically significant hyperkalemia from abnormalities in extrarenal as well as renal potassium homeostasis. Clearly, insulin is an important regulator of potassium uptake by extrarenal tissues, muscle and liver. In the absence of insulin or if there exists resistance to the action of insulin, extrarenal potassium homeostasis will be impaired. Furthermore, insulin lack will, by definition, be associated with a rise in the plasma glucose concentration. The resultant hypertonicity will cause the translocation of water and potassium out of cells into the extracellular compartment (Birhauser *et al.*, 1973; Moreno *et al.*, 1969; Seldin and Tarail, 1949; Tarail *et al.*, 1951), leading to hyperkalemia. Should the insulin deficiency be particularly severe, ketoacidosis may ensue and the metabolic acidemia will exacerbate the hyperkalemia via two mechanisms: (1) Potassium will move out of cells in exchange for hydrogen, which has entered the cell to be buffered by intracellular proteins (Adler and Fraley, 1977; Burnell *et al.*, 1956; Simmons and Avedon, 1959), and (2) the increase in blood hydrogen ion concentration will directly inhibit potassium secretion by the distal tubule and collecting duct (Mahnensmith *et al.*, 1979; Malnic *et al.*, 1971; Soudry *et al.*, 1976). In addition to insulin deficiency, diabetics commonly have a defect in aldosterone secretion (DeFronzo *et al.*, 1979c, 1980b). If present, this will lead to an impairment in potassium uptake by extrarenal tissues (DeFronzo and Bia, 1985) as well as a decrease in renal potassium excretion (Wright and Giebisch, 1985). Further, many diabetics have autonomic neuropathy and diminished circulating epinephrine levels, a hormone known to play an important regulatory role in extrarenal potassium disposal (see subsequent discussion). Finally, renal disease—both glomerular and interstitial—is very frequent in the diabetic individual and will further compromise potassium homeostasis. Thus, disturbances in both the hormonal (insulin, aldosterone, epinephrine deficiency and/or resistance) and metabolic (hypertonicity, acidemia) milieu will conspire to impair both renal and extrarenal potassium metabolism and to predispose the diabetic to the development of clinically significant hyperkalemia.

V. CATECHOLAMINES

Next to insulin, the sympathetic nervous system and circulating catecholamines have been shown to play the most important role in the maintenance of

extrarenal potassium homeostasis. D'Silva (1935) was the first to demonstrate this effect of catecholamines. Following epinephrine injection in cats, he noted a biphasic change in plasma potassium concentration. Within the first 3 min, plasma potassium rose, and this was followed after 5 min by a sustained decrease. Isolation of the liver from the systemic circulation prevented the initial rise, but had no effect on the later decline in plasma potassium level.

Recent studies by DeFronzo *et al.* (1979d, 1981) and Rosa *et al.* (1980) have emphasized the importance of catecholamines in the regulation of overall potassium homeostasis. Infusion of KCl in normal man to raise the plasma potassium concentration by 0.5–1.0 mEq/liter increased potassium excretion 2- to 4-fold. When epinephrine was infused with KCl, the increase in potassium excretion was inhibited by 50–100% (DeFronzo *et al.*, 1979d, 1981; Rosa *et al.*, 1980). Nonetheless, the increment in plasma potassium was less than with KCl alone, and in several individuals no increase in plasma potassium above basal values was observed. From these results, it can be calculated that epinephrine causes a greater than 2-fold increase in the transfer of potasium from the extracellular to the intracellular compartment. This effect of epinephrine on extrarenal potassium metabolism was completely reversed by the simultaneous infusion of propranolol, indicating that the potassium lowering effect was mediated via the β receptor (DeFronzo *et al.*, 1979d, 1981; Rosa *et al.*, 1980). These observations serve to emphasize the important role of catecholamines in the modulation of potassium uptake by extrarenal tissues.

The effect of epinephrine (2 μg/kg·min) on plasma potassium concentration has been studied extensively in the dog (Pettit and Vick, 1974a; Todd and Vick, 1976; Todd *et al.*, 1968; Vick *et al.*, 1972). Although this infusion rate resulted in pharmacological levels of epinephrine, subsequent studies by other investigators using more physiological doses have documented similar results. Thus, epinephrine was found to cause a biphasic response in the plasma potassium concentration similar to that observed by D'Silva (1935). During the initial hyperkalemic phase, an increase in the hepatic venous plasma potassium concentration was observed and this returned to basal values within 5 min. After 5 min, the arterial plasma potassium concentration declined in concert with an increase in the femoral arteriovenous potassium concentration difference. Since neither hepatic nor leg blood flow measurements were made, absolute changes in potassium balance could not be determined. Unfortunately, potassium excretion was not measured, so the contribution of changes in renal potassium excretion to the effect of epinephrine on plasma potassium could not be evaluated. However, the initial hyperkalemic response occurred too early to be accounted for by changes in renal potassium excretion, and the late hypokalemic response occurred when excretion would be expected to be inhibited (DeFronzo *et al.*, 1979d, 1981; Rosa *et al.*, 1980).

Epinephrine causes hyperglycemia by stimulating both hepatic glycogenolysis

and gluconeogenesis. The resultant increase in plasma glucose is known to stimulate insulin secretion. Therefore, it is important to prevent plasma insulin levels from rising when infusing epinephrine. This has been done both by pancreatectomy (Pettit and Vick, 1974a) and somatostatin infusion (DeFronzo *et al.*, 1981). In both experimental designs, the late hypokalemic response to epinephrine remained largely unaltered. Lum and Lockwood (1972) and Lockwood and Lum (1974, 1977) as well as Hiatt *et al.* (1979a,b) also have demonstrated that the rise in plasma potassium concentration following KCl infusion can be ameliorated by epinephrine, that this effect can be reversed by propranolol, and that it occurs in both nephrectomized and pancreatectomized animals.

Clausen *et al.* (1977, 1983, 1986) and Dahl-Hansen and Clausen (1973) have examined the cellular mechanism via which epinephrine enhances potassium uptake. Using the rat soleus muscle preparation, they demonstrated that epinephrine stimulates potassium uptake while at the same time enhancing sodium efflux. Similar results have been reported in rabbit atria (Waddell, 1961), frog sartorius muscle (Hayes *et al.*, 1979), rat diaphragm (Evans and Smith, 1973), and cat skeletal and cardiac muscle (Lockwood and Lum, 1974). Further, the stimulation of muscle potassium uptake by epinephrine can be blocked by propranolol (Clausen and Flatman, 1977; Todd and Vick, 1971; Dahl-Hansen and Clausen, 1973), while propranolol alone enhances the net efflux of potassium from soleus muscle (Dahl-Hansen and Clausen, 1973). This observation may explain the small but significant rise in plasma potassium concentration that occurs following chronic propranolol administration in normal subjects (Pedersen and Kornerup, 1976; Pedersen *et al.*, 1979; Verniory *et al.*, 1976; Waal-Manning, 1976). Using ^{42}K to measure exchangeable potassium, Pedersen *et al.* (1979) showed that the rise in plasma potassium during propranolol therapy could not be explained by net potassium retention. Instead, the hyperkalemia resulted from a shift of potassium from the intracellular to extracellular compartment. More recently, both DeFronzo *et al.* (1979d, 1981) and Rosa *et al.* (1980) have shown that propranolol impairs the disposal of an acutely administered potassium load despite either no change (Rosa *et al.*, 1980) or an augmentation (DeFronzo *et al.*, 1979d, 1981) in renal potassium excretion.

Exercise is another situation in which β-adrenergic blockade has been shown to impair extrarenal potassium metabolism. When normal subjects exercise at 50% of maximum work capacity, a predictable rise in plasma potassium concentration of ~0.5 mEq/liter ensues. If the same exercise is performed following β blockade with propranolol, the peak rise in plasma potassium concentration is 2- to 2.5-fold greater (Carlsson *et al.*, 1978; Castellino *et al.*, 1986). Diabetic subjects are particularly at risk to develop hyperkalemia following β-adrenergic blockade with nonspecific β_1, β_2 antagonists (Fig. 8). In contrast, metoprolol—a specific β_1 antagonist—has no deleterious effect on extrarenal potassium metabolism during exercise (Fig. 8). We have observed similar results in uremic

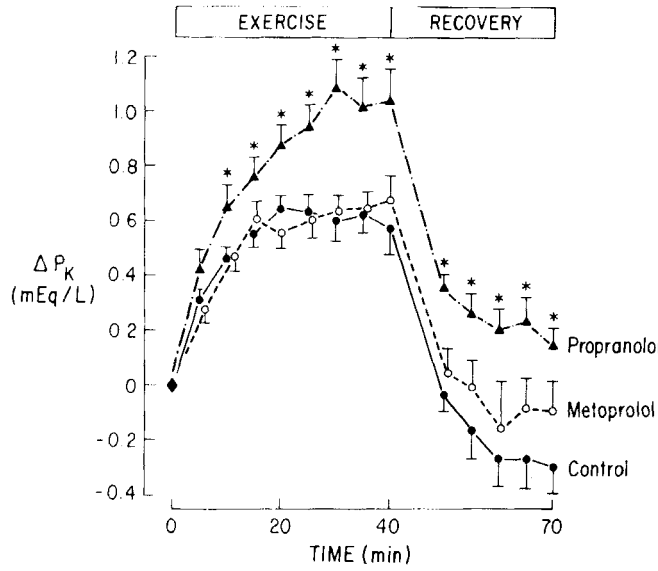

FIG. 8. Effect of exercise without (●——●) and with specific β_1 (metoprolol) (○- - -○) and nonspecific β_1-, β_2- (propranolol) (▲···▲) adrenergic blockade on the plasma potassium concentration in insulin-dependent diabetic subjects. When exercise was performed after propranolol administration, a marked deterioration in potassium homeostasis was observed. In contrast, when subjects exercised following β-adrenergic blockade with metoprolol, extrarenal potassium homeostasis was unaltered.

subjects during exercise. Thus, patients who are being treated with β-blocking agents and who, because of chronic renal failure or the presence of insulin-dependent diabetes mellitus, are predisposed to develop hyperkalemia may benefit from the more selective β_1 blocking agent, metoprolol.

Although the above studies indicate that epinephrine plays an important role in extrarenal potassium homeostasis, they do not define the precise mechanism(s) by which epinephrine promotes the cellular uptake of potassium, since the hormone possesses both α and β_1 as well as β_2 agonistic properties. A number of earlier studies have attempted to answer this question by examining the effect of α- and β-adrenergic agonists and antagonists on potassium metabolism. Before discussing these results, however, it should be pointed out that many of the experimental conditions were unphysiological and employed very large doses of α and β agonists (Todd and Vick, 1971; Vick *et al.*, 1972; Lockwood and Lum, 1974; Lum and Lockwood, 1972; Hiatt *et al.*, 1979a). In addition, huge doses of potassium were administered, resulting in lethal hyperkalemia (plasma K^+ = 15–20 mEq/liter). With these reservations in mind, the schemes summarized in Table I can be envisioned (DeFronzo *et al.*, 1978, 1979a, 1981; Rosa *et al.*,

1980; Pettit and Vick, 1974a; Todd and Vick, 1971; Todd *et al.*, 1968; Vick *et al.*, 1972; Lockwood and Lum, 1974, 1977; Lum and Lockwood, 1972; Hiatt *et al.*, 1979a; Brown *et al.*, 1983; Sternheim *et al.*, 1982).

Infusion of epinephrine or isoproterenol (nonspecific β agonists) causes a decline in plasma potassium concentration that begins within 5 min and persists as long as the catechol is infused (Pettit and Vick, 1974a; Todd and Vick, 1971; Lockwood and Lum, 1974; Lum and Lockwood, 1972; Olsson *et al.*, 1978). When these agents are infused with KCl, the rise in plasma potassium concentration is blunted and cardiac toxicity is prevented. Although epinephrine also has α-agonistic properties, the ability of isoproterenol—a pure β agonist—to induce a similar lowering in the plasma potassium concentration suggests that the effect of these catecholamines is mediated via the β-adrenergic receptor. This conclusion is supported by the inability of phenoxybenzamine, an α blocker, to prevent the hypokalemic effect of β agonists (Todd and Vick, 1971; Lockwood and Lum, 1974; Lum and Lockwood, 1974). Likewise, phenylephrine—an α agonist—causes an increase, not a decrease, in plasma potassium concentration (Todd and Vick, 1971; Lockwood and Lum, 1974). When the specific β_1 agonist, 1-isopropylamino-3-(2-thiazoloxy)-2-propanol (ITP), is infused with KCl, the rise in plasma potassium is similar to that observed with KCl alone (Lockwood and Lum, 1974). In contrast, specific β_2 agonists (salbutamol, solterenol, and terbutaline) all manifest a potassium lowering action (Todd and Vick, 1971; Lockwood and Lum, 1974; Olsson *et al.*, 1978; Struthers and Reid, 1984). These results support the concept that the stimulatory effect of catecholamines on extrarenal potassium uptake is mediated via the β_2 receptor. This conclusion is further supported by results obtained with specific β-adrenergic antagonists. Thus, the specific β_1 antagonist, practolol, cannot reverse the hypokalemic action of β agonists (Lockwood and Lum, 1974; Struthers and Reid, 1984), whereas selective β_2 antagonists such as butoxamine, H 35/25, and ICI 118551 (Pedersen *et al.*, 1979; Brown *et al.*, 1983; Struthers and Reid, 1980, 1984; Berand and Marlin, 1978; Struthers *et al.*, 1983) totally prevent the potassium lowering effect. Pertinent to these observations, salbutamol overdose has been shown to be associated with profound hypokalemia, which is reversed with propranolol treatment (Connell *et al.*, 1982; Obrien *et al.*, 1981).

As discussed earlier, during the initial 3–5 min following epinephrine infusion, a significant rise in the plasma potassium concentraion is observed. This hyperkalemic effect appears to be mediated via the α-adrenergic receptor, since it only occurs with catecholamines that possess α-agonistic activity (D'Silva, 1937; Todd and Vick, 1971; Vick *et al.*, 1972; Lockwood and Lum, 1974, 1977; Lum and Lockwood, 1972; Williams *et al.*, 1984) and can be prevented by α blockers, phenoxybenzamine and phentolamine (Lockwood and Lum, 1974; Lum and Lockwood, 1972; Williams *et al.*, 1984). It is noteworthy that epinephrine, which possesses both α- and β-agonistic activity, causes an early

hyperkalemic response that is followed by a sustained period of hypokalemia. In contrast, phenylephrine—a pure α agonist—elicits a sustained rise in plasma potassium concentration (Todd and Vick, 1971; Williams *et al.*, 1984), suggesting that the α effect of catecholamines such as epinephrine may indeed be persistent, but is overridden by the more potent β_2, potassium lowering action. Recent studies by Williams *et al.* (1984) have clarified the role of the α-adrenergic receptor in extrarenal potassium homeostasis. When phenylephrine, an α agonist, was infused with KCl in man, extrarenal potassium tolerance was impaired. Conversely, phentolamine, an α antagonist, completely reversed the excessive rise in plasma potassium concentration following phenylephrine infusion. From these results one could argue that the deterioration in extrarenal potassium tolerance following β-adrenergic blockade is, in part, due to a relative excess in α-adrenergic tone, More data are needed to establish this hypothesis.

Most recently, Bia *et al.* (1986) have infused physiological doses of β-adrenergic agonists and antagonists in acutely nephrectomized rats to further define the contribution of the β-adrenergic nervous system to extrarenal potassium homeostasis. Epinephrine blunted the rise in plasma potassium concentration following KCl infusion (Fig. 9), and this protective effect could be mimicked by the selective β_2 agonists, salbutamol and terbutaline. In contrast, propranolol (a nonspecific β_1, β_2 blocking agent) and butoxamine (a specific β_2 blocker) exac-

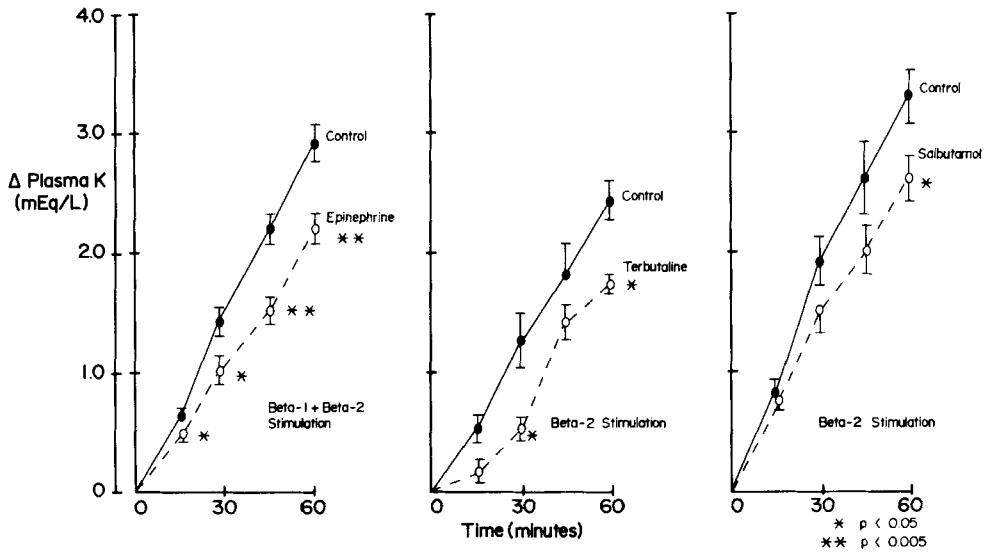

FIG. 9. Effect of various β-adrenergic agonists on extrarenal potassium metabolism in acutely nephrectomized rats. Selective β_2 agonists—as well as nonselective β_1, β_2 agonists—enhanced extrarenal potassium uptake. Selective β_1 agonists had no effect on extrarenal potassium uptake.

erbated the rise in plasma potassium concentration (Fig. 10). The selective β_1 blocking agent, metoprolol, was without effect on extrarenal potassium tolerance (Fig. 10). These alterations in extrarenal potassium disposal could not be explained by changes in circulating plasma insulin, glucose, renin, aldosterone levels, or by alterations in acid–base or hemodynamic status.

Studies in man (DeFronzo *et al.*, 1981; Pedersen *et al.*, 1979) have provided further evidence concerning the important role of the sympathetic nervous system in the regulation of extrarenal potassium metabolism. When basal epinephrine activity is inhibited with propranolol, plasma potassium levels rise and the disposal of an exogenous potassium load is markedly impaired. These deleterious effects on potassium homeostasis are unrelated to changes in U_KV or circulating plasma aldosterone or insulin levels. Additional evidence to support an important role for basal epinephrine levels in the maintenance of normal extrarenal potassium tolerance stems from the work of Bia *et al.* (1982), who demonstrated that the hyperkalemia in chronically adrenalectomized animals could be reversed with physiological replacement of fasting plasma epinephrine levels. Similarly, Sterns *et al.* (1983) have shown that propranolol infusion in dogs causes hyperkalemia by impairing extrarenal potassium disposal.

The important role of epinephrine in extrarenal potassium homeostasis is

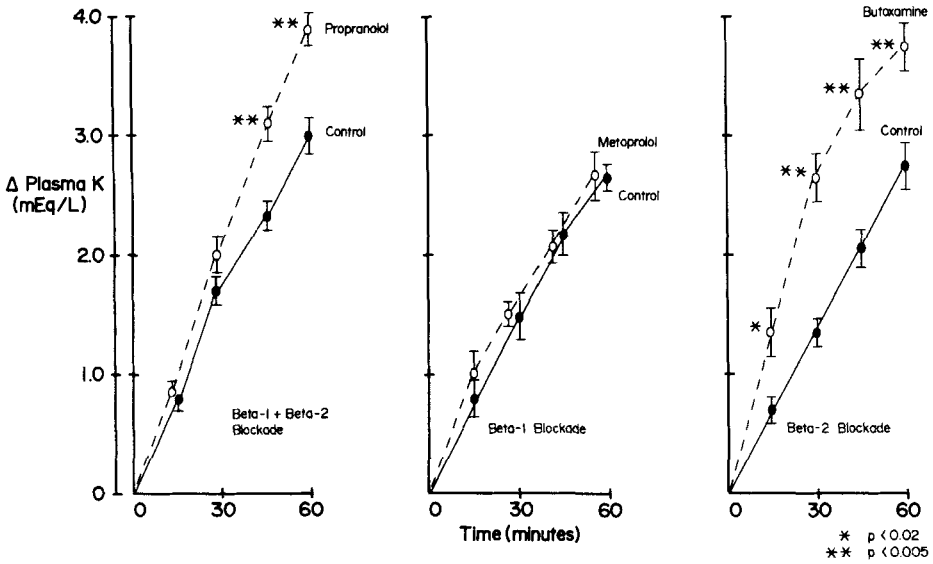

FIG. 10. Effect of various β-adrenergic antagonists on extrarenal potassium metabolism in acutely nephrectomized rats. Selective β_2 antagonists—as well as nonselective β_1, β_2 antagonists—impared extrarenal potassium uptake. Selective β_1 antagonists had no inhibitory effect on extrarenal potassium uptake.

underscored by studies in which the secretion of other hormones (i.e., insulin, aldosterone) involved in extrarenal potassium homeostasis is impaired. Thus, if a state of insulinopenia is superimposed upon adrenalectomy, a marked deterioration in potassium tolerance ensues (DeFronzo *et al.*, 1980b). Similar results have been reported by Silva and Spokes (1981) following combined adrenalectomy and pancreatectomy. Similarly, the combination of adrenalectomy and sympathectomy (produced chemically with 6-hydroxydopamine) also led to a severe impairment in extrarenal potassium tolerance which could be corrected by restoring plasma epinephrine levels to normal. From these investigations has emerged the concept that multiple hormones (catecholamines, mineralocorticoids, insulin) contribute to the normal maintenance of extrarenal potassium metabolism. A deficiency in any single hormone (insulin, epinephrine, aldosterone) will cause only a modest impairment in potassium tolerance as long as the other two remain intact. If, however, the secretion of two or all three potassium regulatory hormones is impaired, a marked defect in extrarenal potassium homeostasis will develop. As discussed earlier, this is most likely to occur in the insulin-dependent (Type I) diabetic who has autonomic neuropathy and the syndrome of hypoaldosteronism.

The cellular mechanisms via which catecholamines regulate extrarenal potassium uptake have recently been reviewed (Clausen, 1983, 1986). The first step in epinephrine action involves its binding to specific adrenergic receptors on the surface cell (Insel, 1984). There are two major classes of adrenergic receptors—α and β—and each class can be further subdivided into at least two subtypes (α_1, α_2, β_1, β_2). The various receptors mediate distinct effects on target tissues, and these effects are initiated by different second messenger systems. The α_1 receptor exerts its effects by altering cytoplasmic calcium concentration, whereas the α_2 receptor works via inhibiting the adenylate cyclase system. β_1 and β_2 receptors mediate their actions by stimulating adenylate cyclase with a resultant increase in intracellular cyclic AMP concentration. As discussed previously, it is the β_2-adrenergic receptor that is primarily involved in the regulation of extrarenal potassium uptake. At present, it is unknown whether the deleterious effect of α-adrenergic stimulation on potassium tolerance is mediated via the α_1 or α_2 receptor. Following the binding of epinephrine (or any other catecholamine with β_2-agonistic activity) to the β_2 receptor, adenylate cyclase is stimulated, and this enhances the conversion of ATP to cyclic 3′,5′-AMP (Flatman and Clausen, 1979; Cheng *et al.*, 1976; Clausen, 1983; Rogers *et al.*, 1977b) (Fig. 11). Cyclic AMP, in turn, activates the enzyme Na^+,K^+-ATPase (Flatman and Clausen, 1979; Cheng *et al.*, 1976; Clausen, 1983; Rogers *et al.*, 1977b), which is felt to be synonymous with or intimately related to the transport system for Na^+ and K^+ (Jorgensen, 1982; Hokin, 1981; Glynn and Karlish, 1975). *In vitro* and *in vivo* studies using skeletal muscle and adipocytes have documented that epinephrine stimulates ^{42}K influx and ^{22}Na efflux, resulting in

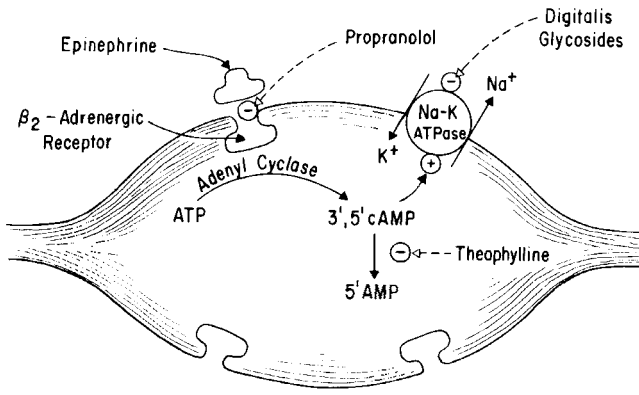

FIG. 11. Schematic representation of the effect of epinephrine on potassium uptake by extrarenal tissues. See text for a detailed discussion.

hyperpolarization of the cell membrane (Flatman and Clausen, 1979; Clausen, 1983, 1986; Clausen and Flatman, 1977, 1980). Consistent with the above biochemical sequence, theophylline potentiates the effect of epinephrine on Na efflux, K influx, and hyperpolarization (Clausen and Flatman, 1977; Clausen, 1983; Rogers *et al.*, 1977b), and the combination of dibutyrl cyclic AMP plus theophylline mimics all of the effects of epinephrine on ion transport (Clausen and Flatman, 1977; Clausen, 1983; Rogers *et al.*, 1977b). The ability of epinephrine to increase [^{3}H]ouabain binding to soleus muscle (Clausen and Hansen, 1977; Clausen and Flatman, 1977) and, conversely, the demonstration that ouabain can block completely the action of epinephrine to stimulate ^{22}Na efflux and ^{42}K influx (Clausen and Flatman, 1977; Clausen and Hansen, 1977) provide further documentation for the cascade of events outlined above. Finally, the effects of epinephrine on the Na^+,K^+-ATPase pump and membrane hyperpolarization can be reproduced by β_2-selective, but not β_1-selective agonists and can be inhibited by β_2-selective, but not β_1-selective antagonists (Flatman and Clausen, 1979). These results of selective β-adrenergic stimulation and blockade on cellular potassium transport are entirely consistent with published data obtained *in vivo* in man and animal.

Several interesting questions concerning the effect of catecholamines on extrarenal potassium metabolism remain unanswered. First, can smaller, more physiological elevations in plasma catecholamine levels enhance cellular potassium uptake? The answer to this question appears to be yes. Thus, when plasma epinephrine levels (800–1200 pg/ml) similar to those observed in surgical stress (Halter *et al.*, 1977) and diabetic ketoacidosis (Christensen, 1974) are reproduced, extrarenal potassium uptake is markedly stimulated. Similar results have been reported in intact and adrenalectomized rats receiving an infusion of

epinephrine designed to simulate plasma epinephrine levels observed during anesthesia (Bia *et al.*, 1982). Finally, and perhaps most importantly, inhibition of basal epinephrine activity with propranolol leads to an elevation of the fasting potassium level and a marked deterioration in the ability of extrarenal tissues to dispose of an exogenously administered potassium load (DeFronzo *et al.*, 1981; Rosa *et al.*, 1980; Pedersen *et al.*, 1978; Sterns *et al.*, 1983). A second unresolved question regards the site(s) of epinephrine-mediated potassium uptake. Muscle clearly plays an important role (Clausen and Flatman, 1979; Todd and Vick, 1971; Vick *et al.*, 1972; Lockwood and Lum, 1974; Evans and Smith, 1973). However, conflicting results concerning the role of the liver have been published (DeFronzo *et al.*, 1979c; Vick *et al.*, 1972; Lockwood and Lum, 1974), and the contribution of other tissues has received little attention. Third, it has yet to be established with certainty whether a feedback loop exists between the plasma potassium concentration and adrenal secretion of catecholamines. Although the ability of epinephrine to augment cellular potassium uptake is firmly established, it is not known whether hyperkalemia can stimulate epinephrine secretion by the adrenal medulla. One study has reported that potassium can stimulate tyrosine hydroxylase, the first enzyme in the catecholamine biosynthetic pathway, in culture adrenal cells (Silberstein *et al.*, 1972). Further, intraarterial KCl injection in cats has been shown to stimulate both epinephrine and norepinephrine release by the adrenal gland. However, the number of animals (3) in this study was small, and neither the dose of KCl nor the resultant plasma potassium concentrations were reported (Vogt, 1952). In a more recent study, DeFronzo *et al.* failed to detect any increase in circulating plasma epinephrine or norepinephrine concentrations when KCl was infused to elevate plasma potassium levels by 0.5–1.0 mEq/liter (DeFronzo *et al.*, 1979d, 1981). Whether larger (1–3 mEq/liter) increments in plasma potassium will stimulate catecholamine release in man remains to be established. It is also possible that mild to moderate elevations in plasma potassium do stimulate catecholamine release, but that local reuptake by the sympathetic nerve terminals obscures a rise in circulating plasma levels (Young and Landsberg, 1977).

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Chapter 14

Diuretics and Potassium

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I. INTRODUCTION

Maintenance of a steep gradient for potassium (K^+) across cell membranes of the body is critical for their normal function. Diuretics can increase renal K^+ elimination substantially and could thereby have widespread secondary effects through K^+ depletion. Study of the effects of diuretics on K^+ elimination therefore has important clinical implications besides throwing light on the fundamental processes that regulate renal K^+ transport.

II. MECHANISMS WHEREBY DIURETICS MAY ALTER RENAL K^+ EXCRETION

The renal control processes which subserve normal K^+ homeostasis have been reviewed extensively (Giebisch, 1981; Stanton and Giebisch, 1981, 1982a; Field and Giebisch, 1985), as have the mechanisms and sites of action of diuretics on Na^+ and fluid reabsorption (Giebisch, 1976; Kelly *et al.*, 1982; Steinmetz and Koeppen, 1984; Kokko, 1984) and K^+ excretion (Giebisch, 1976, 1977). Important mechanisms which have been identified as regulating K^+ excretion and which may be focal points for diuretic action are discussed below.

A. Inhibition of K^+ Reabsorption

K^+ is reabsorbed in the proximal tubule, loop of Henle, and collecting ducts and recycled through the medullary interstitium (Dobyan *et al.*, 1979; Stanton

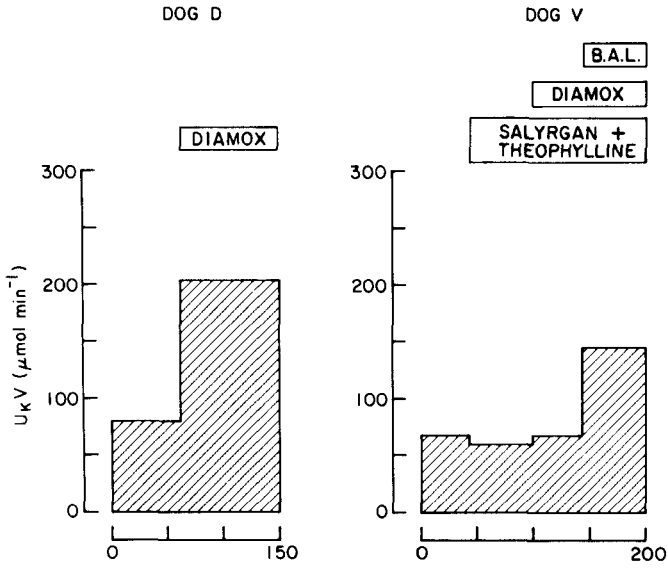

FIG. 1. Clearance data from two conscious dogs showing renal potassium excretion during administration of acetazolamide alone (left) and during administration of acetazolamide with a mercurial diuretic, followed by superimposition of BAL to reverse the action of the mercurial diuretic. [From Berliner *et al.* (1951), with permission.]

and Giebisch, 1982a). In a critical study in the dog, Berliner *et al.* (1951) showed that the kaliuretic action of a carbonic anhydrase inhibitor, acetazolamide, was prevented by inhibition of K^+ transport in more distal segments by a mercurial diuretic. Since these authors also showed that K^+ excretion with acetazolamide could exceed the rate of K^+ filtration, they concluded that decreased proximal K^+ reabsorption could not explain the intense kaliuresis with acetazolamide, which was therefore attributed to enhanced distal secretion. This classic study is also notable for the use of 2,3-dimereapto-1-propanol (BAL) to reverse selectively the action of the mercurial diuretic and thereby to disclose the underlying kaliuretic action of acetazolamide (Fig. 1).

B. Distal Tubule Fluid Flow Rate and Na^+ Reabsorption

The rate of flow of distal tubular fluid is a powerful determinant of tubular K^+ secretion across the physiological range of flow rates (Khuri *et al.*, 1975a). At any given flow rate, tubular secretion is dependent upon the prior K^+ intake, presumably reflecting availability of intracellular K^+ for secretion. Flow could increase K^+ secretion directly by ameliorating the development of a steep axial gradient of distal tubular fluid K^+ concentration. Alternatively, it could act

indirectly by promoting Na^+ reabsorption (Khuri *et al.*, 1975b). However, recent studies have indicated that flow is the more critical variable promoting distal K^+ secretion at physiologically relevant rates of tubular fluid delivery and composition (Wright, 1982). Clearly, increased distal flow could be a powerful mechanism promoting distal K^+ secretion with diuretics acting more proximally (Duarte *et al.*, 1971).

C. Transepithelial Electrical Potential

The distal tubule and cortical collecting ducts normally maintain steep transepithelial potential differences (Wright, 1971). Since these are lumen negative, their magnitude could determine distal K^+ and H^+ ion secretion. Indeed, the increased K^+ and H^+ excretion that accompanies the infusion of Na^+ with poorly reabsorbed anions has been related to enhanced distal lumen electronegativity (Clapp *et al.*, 1962).

D. Tubular Fluid pH

Metabolic or respiratory acidosis normally inhibits distal K^+ secretion whereas alkalosis enhances it; overall, a close dependency of distal K^+ secretion on tubular fluid pH has been found during a variety of short-term changes in acid–base balance (Malnic *et al.*, 1971). While this effect of pH can be offset by accompanying changes in distal flow and Na^+ delivery (Stanton and Giebisch, 1982b), it remains an important determinant of K^+ excretion in settings of altered HCO_3^- delivery or acid–base status.

E. Intracellular K^+ Concentration

Dietary K^+ deprivation or acute metabolic acidosis reduces the intracellular K^+ ion activity measured in the proximal tubule cells of the rat *in vivo* (Cemerikic *et al.*, 1982). In studies of single distal tubules of *Amphiuma*, cellular K^+ content has been related to the peritubular (plasma) K^+ concentration and the prevailing K^+ intake of the animals (Oberleitner and Giebisch, 1981). Increased cellular K^+ concentration will increase the chemical gradient for K^+ transfer from cell to lumen and thereby promote distal K^+ secretion.

F. Extrarenal Hormones

Aldosterone increases K^+ secretion by collecting duct epithelium, although recent studies have highlighted the importance of distal delivery in modulating the kaliuretic action of aldosterone (Field and Giebisch, 1985). An important

action of antidiuretic hormone (ADH) in promoting distal K^+ secretion has been documented in experiments in which the hormone's usual effects on distal fluid delivery were controlled by microperfusion of the superficial distal tubule of the rat (Field *et al.*, 1984b).

III. EFFECTS OF DIURETICS ON NEPHRON K^+ TRANSPORT

A. Water Diuresis

Water diuresis increases K^+ excretion only transiently, if at all, despite large increases in urine flow rate and delivery of fluid to distal segments (Wilcox *et al.*, 1984b). Compared to water diuresis, saline infusion elicits a greater kaliuresis despite a lesser diuretic effect (Wilcox *et al.*, 1984a). Studies in the Brattleboro rat have revealed that inhibition of ADH release accounts for the absence of a flow-related increase in distal K^+ secretion during water loading (Field *et al.*, 1984b) (Fig. 2).

B. Osmotic Diuresis

By contrast to water diuresis, in classic studies, Berliner (1961) and Mudge *et al.* (1950) demonstrated that large increases in K^+ excretion occur during infusions of hypertonic solutions or cellular dehydration which are independent of urine flow rate. This kaliuretic response was originally attributed to increased cellular K^+ concentration, since infusion of hypertonic solutions increased K^+ excretion more than did equally diuretic infusions of isotonic solutions (Mudge *et*

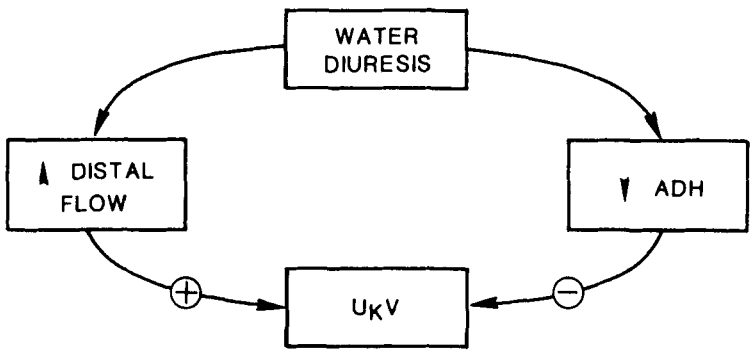

FIG. 2. Diagrammatic representation of contrasting effects of a water load on potassium excretion. ADH, antidiuretic hormone release.

al., 1950; Berliner, 1961; Rabinowitz and Gunter, 1972). However, release of ADH evoked by cellular dehydration may be an additional critical factor promoting K^+ secretion. Osmotic diuretics also increase the delivery of K^+ to the distal tubule as well as increasing the flow of tubular fluid through this segment (Malnic *et al.*, 1966). Presumably osmotic diuretics inhibit K^+ reabsorption in the proximal tubule and loop of Henle. At equivalent diuretic doses, Na^+-containing osmotic diuretics stimulate distal K^+ secretion to a greater extent than nonelectrolytes such as mannitol (Malnic *et al.*, 1966). This effect of Na^+ was attributed to an additional component of distal K^+ secretion related to tubular Na^+ reabsorption, probably secondary to enhanced luminal electronegativity (Clapp *et al.*, 1962). The sharp increase in renal K^+ excretion with osmotic diuretics is therefore to be expected from their disparate actions, which together ensure an enhanced distal K^+ delivery and an enhanced distal K^+ secretion (Fig. 3).

C. Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors such as acetazolamide induce an intense kaliuresis. Indeed, at "therapeutic" doses, these agents are severalfold more kaliuretic than other diuretics and have the unique distinction of promoting at least as great an increase in excretion of K^+ as Na^+ (Mudge, 1980). In a landmark study, Berliner *et al.* (1951) showed that this kaliuretic action was due to enhanced distal K^+ secretion. This cannot be attributed to direct competition for

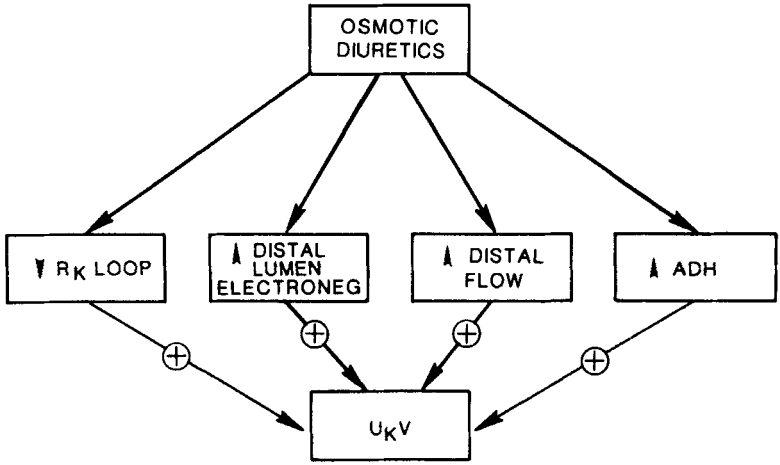

FIG. 3. Diagrammatic representation of the effects of an osmotic diuresis on potassium excretion. R_K loop, potassium reabsorption in the loop segment; ADH, antidiuretic hormone release.

secretion between K^+ and H^+ as suggested by Berliner *et al.* (1951), since acetazolamide administered to the rat enhances the distal delivery of bicarbonate (HCO_3^-) sufficiently to *increase* distal H^+ secretion (Malnic *et al.*, 1972). Rather, the increased distal K^+ secretion during carbonic anhydrase inhibition could relate to increased distal fluid flow (Khuri *et al.*, 1975a), increased distal lumen electronegativity, and increased distal tubule fluid pH (Malnic *et al.*, 1971). Despite the impressive short-term increases in K^+ excretion induced by acetazolamide, extensive studies by Maren and his colleagues (Maren *et al.*, 1954a,b) established that repeated administration of the drug led to a sharply diminished renal K^+ loss over the first few days of drug administration.

D. Loop Diuretics

A major site of action of furosemide, bumetanide, ethacrinic acid, and indacrinone is the ascending limb of the loop of Henle (Burg and Green, 1973; Field *et al.*, 1984a; Hropot *et al.*, 1985). These "loop diuretics" inhibit the cotransport of Cl^- with Na^+ and K^+ at this segment, thereby increasing delivery of fluid, Na^+, and K^+ to the distal tubule. Inhibition of K^+ reabsorption in the loop of Henle was shown first in microperfusion studies in the rat by Morgan *et al.* (1970) and was confirmed recently for indacrinone (Field *et al.*, 1984a; Fig. 4). Micropuncture studies have shown that furosemide can also inhibit fluid reabsorption in the proximal tubule (Suki *et al.*, 1965; Knox *et al.*, 1969),

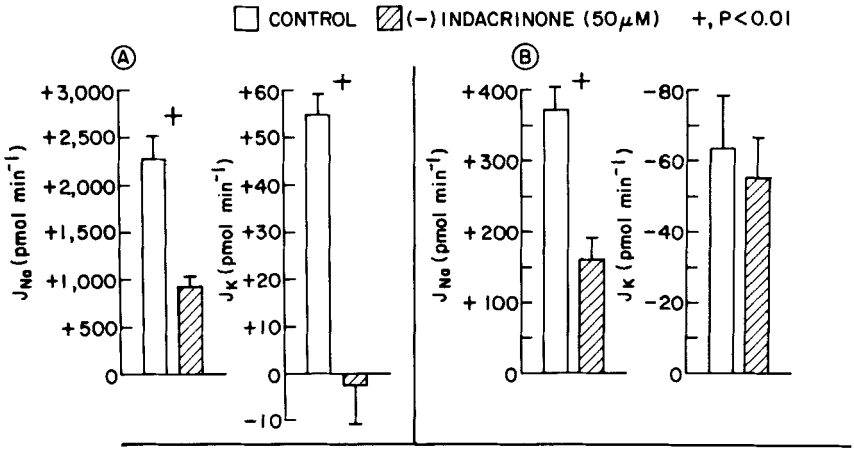

FIG. 4. Microperfusion studies of the loop of Henle (A) and the superficial distal tubule (B) in rats showing net fluxes of sodium and potassium in control tubules and those perfused with + indacrinone. $P < 0.01$. [After Field *et al.* (1984a), with permission.]

although in other studies, proximal reabsorption can even be enhanced (Dirks *et al.*, 1966), probably depending on the changes in extracellular fluid volume (ECV) induced by the diuretic action (Brenner *et al.*, 1969; Burke *et al.*, 1972).

Free-flow micropuncture studies in the rat have disclosed two nephron sites which contribute to increased K^+ elimination during furosemide administration (Duarte *et al.*, 1971; Giebisch, 1976, 1977; Hropot *et al.*, 1985). First, the finding that fractional K^+ delivery to the early distal tubule (ED) can be increased severalfold by furosemide implies decreased K^+ reabsorption more proximally, presumably in the loop of Henle (Hropot *et al.*, 1985; Fig. 5). Second, furosemide administration increases K^+ secretion by the superficial distal tubule, accompanied by increased Na^+ delivery and tubular fluid flow through this segment. Accordingly, furosemide augments delivery of K^+ to the distal tubule and enhances distal fluid flow and K^+ secretion of this segment.

To separate any direct effects of loop diuretics on distal K^+ transport from indirect actions due to changes in fluid or Na^+ delivery or systemic and endocrine consequences of the diuresis, the superficial distal tubule of the rat has been profused *in vivo* with diuretic-containing perfusates (Velasquez and Wright, 1983; Field *et al.*, 1984a). These two studies concur in demonstrating that the loop diuretics indacrinone (Fig. 4) or furosemide inhibit net reabsorptive fluxes of Na^+ and Cl^- without modification of the net secretory flux for K^+. Accordingly, the enhanced distal K^+ secretion observed during free-flow studies must represent an indirect effect of loop diuretics, as suggested earlier by Duarte *et al.* (1971).

In papillary microcatheterization studies performed in the rat by Sonnenberg

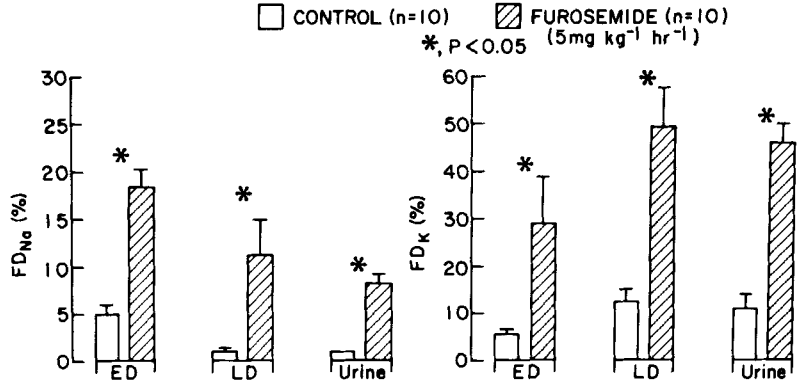

FIG. 5. Mean (±SEM) values from free-flow micropuncture studies showing fractional deliveries of Na^+ (left) and K^+ (right) to the early distal (ED) and late distal (LD) tubule and to the urine in control rats and those receiving intravenous furosemide. [After Hropot *et al.* (1985), with permission.]

and colleagues (Sonnenberg, 1978; Wilson *et al.*, 1983), furosemide was shown to alter the fluxes of fluid, Na^+, and K^+ through the medullary collecting duct (Fig. 6). While the fractional reabsorption of Na^+ and water was reduced or abolished, fractional secretion of K^+ was augmented. These authors argued that these changes were not related primarily to the effects of the diuretic on the loads of fluid and ions presented to this segment (Fig. 7), although they could be a consequence of alterations in the medullary interstitial environment. Regardless of the mechanisms, these results are especially important because they show major changes in K^+ secretion occurring with furosemide in the most terminal nephron segment, with no possibilities for compensatory adjustments in K^+

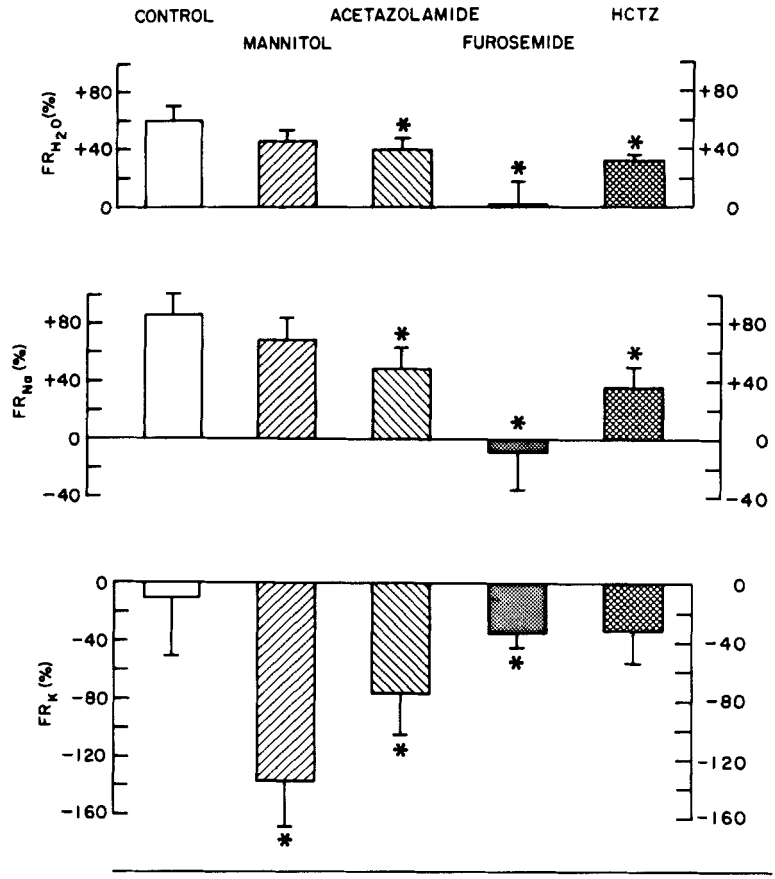

FIG. 6. Data from papillary microcatheterization studies in the rat showing fractional reabsorption of water, sodium, and potassium in control rats and those treated with mannitol, acetazolamide, furosemide, and hydrochlorothiazide. *, $P < 0.05$. [After Sonnenberg (1978) and Wilson *et al.* (1983), with permission.]

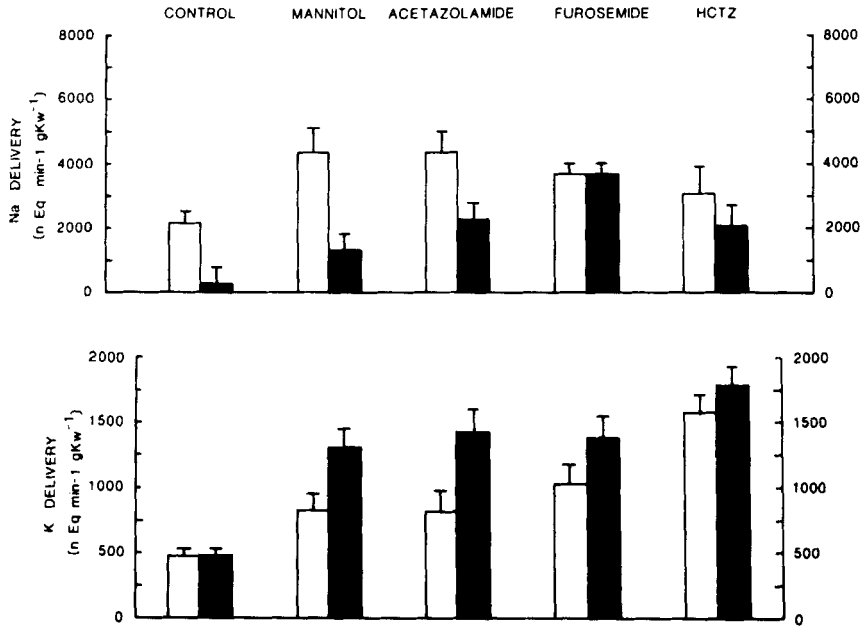

FIG. 7. Mean (±SEM) values from papillary microcatheterization studies in the rat showing absolute rates of sodium and potassium delivery to the beginning (open bars) and end (solid bars) of the medullary collecting ducts. Studies were performed under hydropenic control conditions and during intravenous infusion of mannitol (0.3 g and 0.3 g hr^{-1}), acetazolamide (0.5 mg and 0.5 mg hr^{-1}), furosemide (1.5 mg and 1.5 mg hr^{-1}), and hydrochlorothiazide (2 mg kg^{-1} and 2 mg kg^{-1} hr^{-1}). [After Sonnenberg (1978) and Wilson *et al.* (1983), with permission.]

transport to occur downstream. Therefore the effects of furosemide on this segment must be translated directly into increased renal K^+ losses.

It is apparent that the kaliuretic effects of loop diuretics can be attributed to a concordance of actions along the nephron. Decreased reabsorption of K^+ in the loop (and perhaps more proximally) delivers increased K^+ loads more distally. However, the major effects of the loop diuretics are due to increased secretion of K^+ in the distal nephron and collecting ducts; much of the augmented distal K^+ secretion is secondary to enhanced fluid and Na^+ delivery, but additional effects can occur in the terminal nephron segments.

E. Thiazide Diuretics

The administration of a thiazide diuretic increases Na^+ and K^+ excretion abruptly, albeit not to the levels seen with loop diuretics. Clearance (Mudge, 1980) and micropuncture (Kunau *et al.*, 1974; Windhager and Constanzo, 1978)

studies concur in showing that the early distal tubule is a major site at which thiazide diuretics inhibit Na^+ reabsorption. Free-flow micropuncture studies have disclosed also an increased distal K^+ secretion during thiazide administration in the rat (Hropot *et al.*, 1985; Fig. 8). Like the loop diuretics, thiazides augment the delivery of K^+ to the early distal tubule. Of considerable interest is a finding that addition of thiazide to perfusates of the superficial distal tubule reduces Na^+ and Cl^- reabsorptive fluxes to a greater extent than that achieved with furosemide; yet, thiazide did not modify the K^+ secretory flux (Velasquez and Wright, 1983). This implies that, as with the loop diuretics, enhanced K^+ secretion with thiazides cannot be attributed to a direct effect of the diuretics on the tubular epithelium. Velasquez and Wright (1983) demonstrated also that addition of a thiazide to a maximal dose of furosemide perfusing the distal tubule did not modify the inhibition of Na^+ or Cl^- reabsorptive fluxes, nor did it disclose an action on K^+ secretion. These interesting results imply that thiazides and furosemide inhibit a similar distal transport mechanism for Na^+ and Cl^-, but that neither class of diuretics affects distal K^+ secretion directly.

In microcatheterization studies of the rat renal papilla, thiazides were shown to inhibit the reabsorptive fluxes of Na^+ and fluid in the medullary collecting ducts, yet, they did not have significant effects on the secretory K^+ flux (Sonnenberg, 1978; Wilson *et al.*, 1983; Figs. 6 and 7).

The micropuncture studies, therefore, inducate rather similar actions of thiazides and furosemide in the distal tubule and collecting duct system. The more intense kaliuretic action of the loop diuretics may be related to their greater effects in promoting increased fluid flow and Na^+ delivery through these segments and perhaps to additional actions in the medullary collecting ducts.

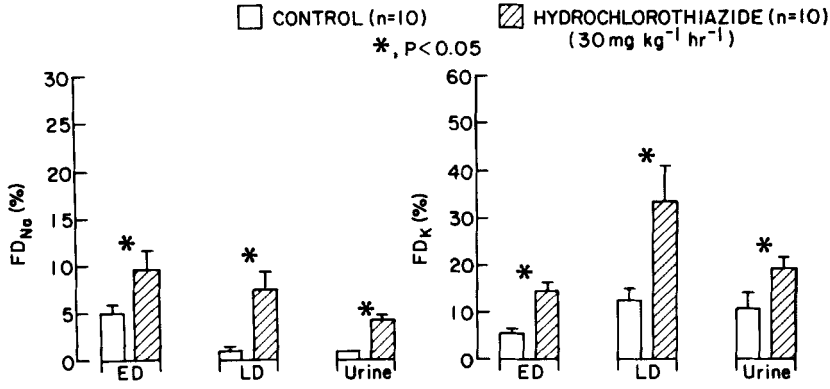

FIG. 8. Mean (±SEM) values from free-flow micropuncture studies showing fractional deliveries of Na^+ (left) and K^+ (right) to the early distal (ED) and late distal (LD) tubules and to the urine in control rats and those receiving intravenous hydrochlorothiazide. [After Hropot *et al.* (1985), with permission.]

F. K^+ Sparing Diuretics

While high concentrations of amiloride inhibit transport at several nephron sites, at therapeutically relevant concentrations the distal nephron and collecting ducts have been identified as the major sites of action of amiloride, triamterine, and spironolactone (Fig. 9; Duarte *et al.*, 1971; Gross and Kokko, 1977; Giebisch, 1978; O'Neil and Boulpaep, 1979; Koeppen *et al.*, 1983; Wingo, 1985; Hropot *et al.*, 1985). These studies have shown that during mineralocorticosteroid stimulation, the addition of amiloride or spironolactone to luminal fluid perfusing isolated cortical collecting tubules leads to an abrupt attenuation, or even reversal, of the lumen-negative transepithelial voltage. There is a concurrent sharp reduction in Na^+ reabsorption and inhibition or abolition of K^+ secretion. These studies suggest that a major mechanism of action of amiloride is to prevent luminal Na^+ entry; the ensuing reduction in the favorable electrical gradient for K^+ secretion from cell to lumen could explain the inhibition of K^+ transport observed. In contrast, Wingo (1985) showed that addition of amiloride to cortical collecting tubules dissected from adrenalectomized rabbits led to a voltage-independent inhibition of K^+ secretion.

Free-flow micropuncture studies have shown that amiloride and triamterene decrease or abolish the normal steep axial rise in K^+ concentration in tubular fluid in the distal nephron and prevent the rise in fractional delivery of K^+ in this segment (Fig. 9; Duarte *et al.*, 1971; Hropot *et al.*, 1985). Amiloride and triamterine can abolish the increase in distal K^+ secretion induced by thiazide or loop diuretics (Hropot *et al.*, 1985). By controlling distal tubule fluid flow rate

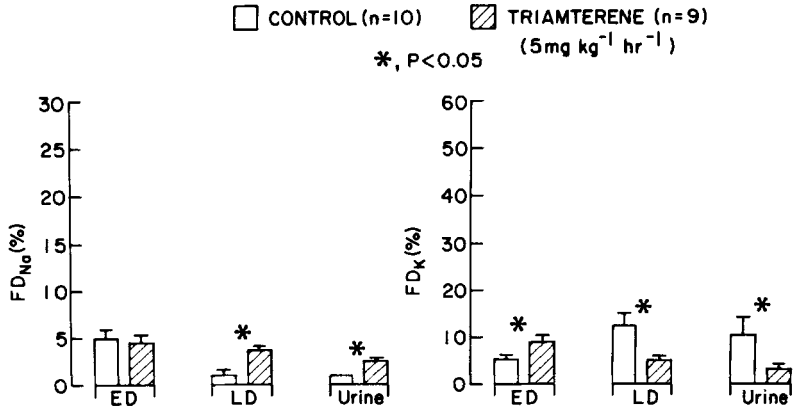

FIG. 9. Mean (±SEM) values from free-flow micropuncture studies showing fractional deliveries of Na^+ (left) and K^+ (right) to the early distal (ED) and late distal (LD) tubule and to the final urine in control rats and those receiving intravenous triamterene. [After Hropot *et al.* (1985), with permission.]

and ionic composition with microperfusion, Velasquez and Wright (1983) found that amiloride, unlike furosemide and thiazides, inhibited the K^+ secretory flux without effects on Na^+ or Cl^-. Moreover, this effect on K^+ transport was quite distinct from that of thiazides and furosemide, since it was additive to maximal responses achieved by these other agents.

G. Cardiac Glycosides

Cardiac glycosides administered to the dog or rat can increase the excretion of Na^+ and K^+ (Cade *et al.*, 1961; Strieder *et al.*, 1974), whereas when added to the perfusate of an isolated rat's kidney, the can inhibit K^+ excretion (Bowman *et al.*, 1973). A direct stimulation of K^+ secretion and inhibition of Na^+ reabsorption was shown by Duarte *et al.* (1971) in distal tubule microperfusion studies in the rat, which was attributed to inhibition of the active reabsorptive K^+ pump in the luminal cell membrane. Perhaps in other situations in which cardiac glycosides inhibit K^+ secretion, there is a critical fall in cellular K^+ concentration due to inhibition of peritubular K^+ uptake, secondary to inhibition of Na^+,K^+-ATPase.

IV. POTASSIUM BALANCE DURING DIURETIC ADMINISTRATION

A paradox is apparent between the predictable kaliuretic response to diuretics seen in short-term studies in both man and animals and the absence of profound cellular K^+ depletion during prolonged periods of diuretic administration. In a review of studies in which measurements of serum and total body K^+ were made in diuretic-treated hypertensive patients, Harrington *et al.* (1982) found little evidence of cellular K^+ depletion even during prolonged diuretic therapy. This contrast of short- and long-term actions is particularly clear for acetazolomide, which is the most potent kaliuretic diuretic in short-term studies. Yet, patients receiving the drug for a prolonged period for treatment of glaucoma do not show evidence of K^+ depletion or a fall in serum K^+ concentration (S_K) (Spaeth, 1967). While the S_K of patients receiving thiazide or loop diuretics for hypertension tends to fall by about 0.5 mmol liter^{-1}, changes in total body K^+ are often insignificant and, for the combined series reported by Harrington *et al.* (1982), represented less than a 5% reduction. In contrast, in patients with severe edema, hyperaldosteronism, or alkalosis, or those treated with a combination of kaliuretic diuretics, more serious K^+ deficits may be incurred. Moreover, concern about even modest degrees of hypokalemia seen during diuretic therapy for hypertension may be appropriate (Kaplan, 1984) in view of the failure of

diuretics to reverse the excess cardiovascular mortality associated with hypertension in most clinical trials (Morgan *et al.*, 1980) and the relationship between diuretic-induced falls in S_K and ventricular ectopic activity (Hollifield, 1984). Moreover, repletion of a K^+ deficit can lower the blood pressure of diuretic-treated hypertensive subjects (Kaplan *et al.*, 1985), although in animal studies, high K^+ intakes have been found to blunt the natriuretic action of hydrochlorothiazide (Olesen, 1982). The effects of diuretics on K^+ balance are of interest also because of the unusual combination that they evoke of increased distal fluid delivery and increased aldosterone secretion. These two factors normally offset one another in many physiological settings (Sealey and Laragh, 1974; Field and Giebisch, 1985).

Prolonged water diuresis in the rat induced by diabetes insipidus leads to a modest degree of negative K^+ balance which can be reversed by vasopressin administration (Mohring *et al.*, 1974). In contrast, prolonged administration of water and ADH leads to K^+ losses (Lowance *et al.*, 1972). Severe total body K^+ deficits are seen in clinical settings such as diabetic ketoacidosis in which ADH levels may be high in the presence of an ongoing osmotic diuresis.

Maren and colleagues have undertaken an extensive series of balance studied in dogs given acetazolamide for prolonged periods (Maren *et al.*, 1954a,b). There is normally only a short period of negative K^+ balance; however, the kaliuretic action of acetazolamide was diminished, but not extinguished after four doses (33 mg kg^{-1}; 8 hourly intervals), but a modest kaliuretic response to the drug persisted and outlived the extinction of the natiuretic action of the diuretic. Inspection of these detailed data indicates the K^+ balance may be maintained during repeated daily doses of acetazolamide, despite some continuing kaliuretic action of the drug, because of a reduction in K^+ excretion in the drug-free periods which follow the diuresis and kaliuresis.

While administration of acetazolamide to patients with glaucoma normally causes little or no K^+ depletion (T. H. Maren, personal communication), its administration to patients with severe cardiac failure or cirrhosis can induce a prominent loss of K^+ in the urine which may exceed even the loss of Na^+ (Leaf *et al.*, 1954). Presumably, enhanced distal tubule avidity for Na^+ in these edematous states can promote ongoing reabsorption of Na^+ and secretion of K^+ in the distal nephron such that K^+ becomes the dominant cation to accompany HCO_3^- in the urine in these clinical settings of intense salt retention.

K^+ depletion can be a concern during diuretic therapy (Kaplan, 1984). Therefore, it is important to define the factors which regulate Na^+ and K^+ balances during diuretic administration to maximize the efficacy and decrease the toxicity of prolonged diuretic therapy. Kahn *et al.* (1980) found that administration of a high salt intake to dogs prevented the development of negative balances for Na^+, K^+, and Cl^- during 4 days of furosemide administration. This was a striking finding in view of the observation of Earley and Martino (1970) that a

high salt intake clearly potentiated the acute natriuretic response to a loop diuretic in man. Moreover, ECV expansion augments the action of furosemide on Na^+ reabsorption in the loop of Henle (Gutsche *et al.*, 1980). In contrast, during a low salt intake, furosemide administered to conscious dogs led to negative balances for Na^+, K^+, and Cl^- (Kahn *et al.*, 1980). Further studies in the rat in which widely varying electrolyte intakes were provided showed that restriction of dietary Cl^- intake was more effective than restriction of Na^+ in provoking Na^+ and K^+ depletion during furosemide administration (Kahn *et al.*, 1983). No correlation was apparent in these animal studies between diuretic-induced changes in K^+ balance and aldosterone secretion. However, the diets produced major disturbances in the acid–base status of the animals which could have exerted an overwhelming influence on K^+ balance (Malnic *et al.*, 1971).

We provided a closely regulated diet to normal human subjects for 8-day periods and after they had equilibrated to the electrolyte intake, administered furosemide (40 mg/day) for 3 or 4 days. In the first protocol, we studied the effects of variation in Na^+ intake on Na^+ and K^+ balances during furosemide administration (Wilcox *et al.*, 1983; Kelly *et al.*, 1983; Wilcox *et al.*, 1984b). Two independent mechanisms were disclosed which limited Na^+ depletion during diuretic administration. The first, termed diuretic extinction, was apparent during a reduced Na^+ intake (20 mmol/day) as a progressive reduction in the acute natriuretic response to the diuretic. The second, termed diuretic compensation, was apparent during higher salt intakes (270 mmol/day) as a compensatory reduction in Na^+ excretion in the 18 hr following the completion of the acute diuresis. This compensation was sufficient to restore neutral Na^+ balances on each day before the next dose of the diuretic was administered. Accordingly, negative balance for Na^+ and loss of body weight was seen only during the low salt intake where renal compensation could not offset the negative salt balance induced by the prior action of the diuretic.

In another protocol, we examined the effects of dietary salt intake and the renin–angiotensin–aldosterone (RAA) system on K^+ homeostasis during furosemide administration (Wilcox *et al.*, 1984b). During a liberal salt intake which diminished aldosterone levels, the acute kaliuretic action of furosemide was followed by a precise renal compensation for K^+, similar to that seen for Na^+, during which K^+ excretion was reduced for about 18 hr after each dose of the diuretic (Fig. 10). Accordingly, K^+ balance was neutral during the high salt intake. In contrast, during the low salt intake which promoted aldosterone secretion, there were ongoing K^+ losses throughout the 24 hr of each day, manifest both during the acute diuresis and subsequently. Accordingly, renal compensation for K^+ was abolished during the low salt intake and K^+ balance was negative for each subject (Fig. 10). Since the low salt intake stimulated the RAA axis and changes in K^+ balance correlated closely with changes in plasma aldosterone concentration, we concluded that aldosterone could have mediated

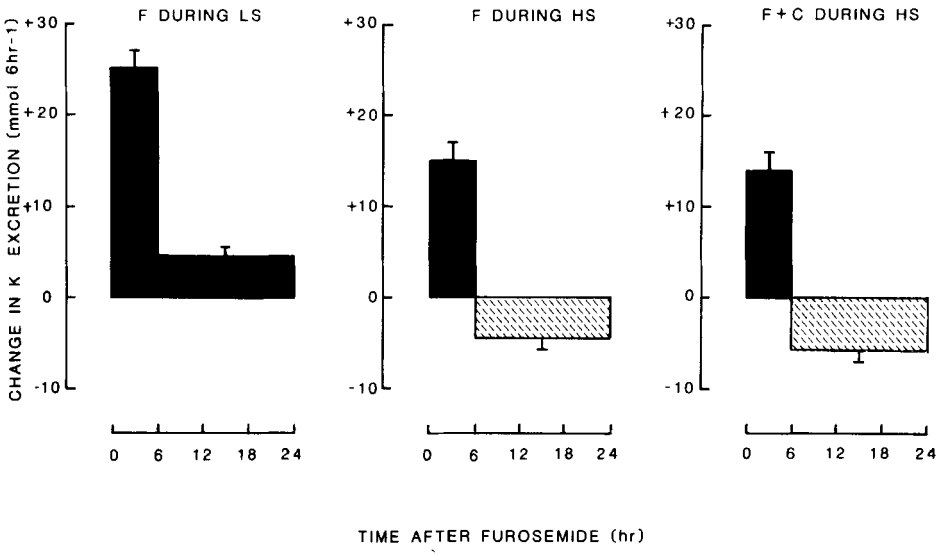

FIG. 10. Mean (±SEM) values for changes in K^+ excretion as a function of time after administration of 40 mg of furosemide intravenously to 6 normal human subjects consuming a low salt (LS; 20 mmol Na^+ day^{-1}) or high salt (HS; 270 mmol Na^+ day^{-1}) intake. In a third protocol, those subjected received captopril (F + C; 25 mg 6 hr^{-1}) during the high salt intake. Periods of increased K^+ excretion are shown in solid bars and decreased K^+ excretion with cross-hatching. [After Wilcox *et al.* (1984b), with permission.]

the effects of dietary salt restriction on diuretic-induced K^+ balance. To probe further the role of the RAA system in regulation of K^+ balance at higher levels of salt intake, we administered captopril (25 mg each 6 hr) to 6 normal subjects consuming a high salt intake (Kelly *et al.*, 1983). Captopril prevented the furosemide-induced increases in plasma angiotensin II and aldosterone concentrations, but did not alter K^+ balance (Fig. 11). This suggests that more modest increases in plasma aldosterone concentration, as accompany furosemide administration on a liberal salt intake, do not augment K^+ excretion sufficiently to overcome the compensatory responses which normally prevent K^+ depletion. Indeed, Ram *et al.* (1981) observed that reducing dietary salt intake from about 200 to about 75 mmol/day ameliorated the fall in exchangeable K^+ in subjects receiving diuretics for hypertension.

The abrupt reduction in central blood volume produced by administration of diuretics (DiBona and Sawin, 1985) may increase ADH release. Since ADH can modulate distal K^+ secretion (Field *et al.*, 1984b), we contrasted the kaliuretic response to furosemide in hydropenic and maximally water-diuretic subjects (Wilcox *et al.*, 1984b). Whereas the water load augmented the diuretic response to furosemide, the short-term naturiuretic response was unchanged, and the acute

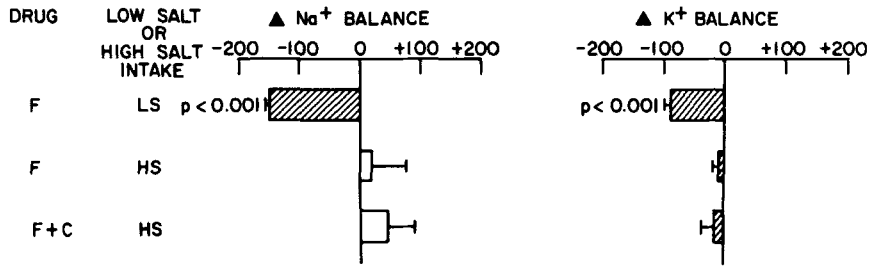

FIG. 11. Mean (±SEM) values for changes in Na^+ and K^+ balances over 3 days of furosemide administration in 6 normal subjects consuming a low salt (LS) or high salt (HS) intake or a high salt intake given with captopril (F + C 25 mg 6 hr^{-1}). Note that a significant negative balance for Na^+ and K^+ developed during furosemide administration with a low salt intake, but no significant changes in balances occurred during high salt intake with or without captopril. [After Wilcox *et al.* (1984b), with permission.]

kaliuresis was actually diminished by 40%. The water load did not modify the furosemide-induced increase in plasma aldosterone levels (Fig. 12). We concluded that up to 40% of the acute K^+ losses induced by a loop diuretic in normal human subjects might be accounted for by release of ADH. Clearly, water loading can have other effects both within and without the kidney which might contribute to the inhibition of furosemide-induced kaliuresis; therefore, further studies will be required with more specific inhibitors to probe this relationship between ADH and K^+ loss. Nevertheless, the observation could have

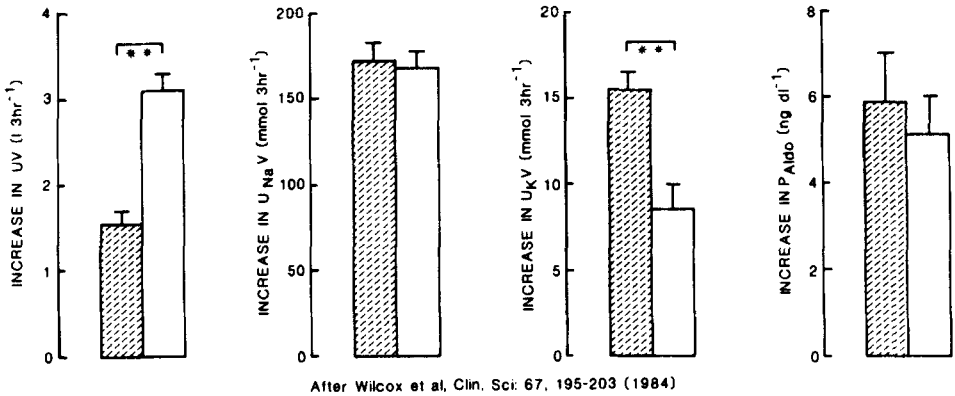

FIG. 12. Mean (±SEM) values for increases in urine flow rate, Na^+ and K^+ excretion, and plasma aldosterone concentration over 5 hr after administering furosemide (40 mg intravenously) to 6 normal human subjects during hydropenia (diagonal hatching) or during an ongoing water load (20 ml kg^{-1} of body weight, with replacement of urinary losses). **, $P < 0.01$. [After Wilcox *et al.* (1984b), with permission.]

important clinical consequences, since patients with severe edema or with the syndrome of inappropriate ADH release have high levels of ADH which could underlie the serious K^+ depletion that can accompany diuretic administration in these conditions.

In summary, the kaliuretic response to diuretics depends on the class of drug and its dosage. Kaliuresis is promoted by severe dietary salt restriction or by water loading which suggests important modulation by aldosterone and AHD. However, despite the short-term K^+ losses, K^+ balance is normally well maintained during prolonged diuretic therapy. Two independent mechanisms have been identified which can limit losses of K^+, Na^+, and fluid; these are extinction of the diuretic response and a compensatory period of retention in the drug-free, postdiuretic phase. Potassium balance during administration of loop diuretics can depend on the level of salt intake and probably relates to the prevailing level of aldosterone secretion. However, the factors that limit distal fluid delivery or distal K^+ secretion in the compensation period and that are therefore critical for maintenance of K^+ homeostasis during repeated diuretic administration remain to be defined.

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Chapter 15

Pathogenesis and Pathophysiological Role of Hypoaldosteronism in Syndromes of Renal Hyperkalemia

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Hyperkalemia of chronic duration (months to years) almost invariably reflects an impairment of potassium handling by the kidney, usually an impairment of the potassium secretory mechanism of the distal nephron, chiefly the collecting tubules and ducts. This is so-called renal hyperkalemia, a term that indicates the primary role of the kidney in the pathophysiology of the hyperkalemic state. Unless the severity of the disturbance is progressing rapidly, the external balance of potassium is not positive or not discernibly so, and affected patients appear to be in a steady state in which dietary intake of potassium is balanced by excretion of potassium in urine and stool. Nevertheless, in patients with persisting hyperkalemia, however mild, the underlying impairment of the renal potassium excretory mechanism can be recognized by the finding of an abnormally low ratio of urine potassium excretion to plasma potassium concentration—i.e., the renal

TABLE I
PATHOPHYSIOLOGICAL MECHANISMS POTENTIALLY CAUSING RENAL HYPERKALEMIA[a]

Decreased fluid flow through collecting tubules
Decreased availability of luminal Na^+ for reabsorption in collecting tubules
Decreased "avidity" of collecting tubules for Na^+
Hypoaldosteronism
Decreased Na^+,K^+-ATPase activity
Primary enhancement of chloride reabsorptive avidity of the collecting tubule
Decreased intracellular K^+ concentration in collecting tubules
Hypoaldosteronism
Decreased Na^+,K^+-ATPase activity
Metabolic acidosis
Decreased luminal membrane potassium permeability in collecting tubules
Hypoaldosteronism
Decreased luminal fluid pH in collecting tubules

[a] Reproduced from Sebastian *et al.* (1985) with permission.

clearance of potassium is abnormally low—whether the steady-state urinary excretion rate of potassium is normal or subnormal.

The pathophysiological mechanisms potentially causal of renal hyperkalemia are summarized in Table I. This summary is a logical extrapolation of current knowledge of the normal physiology of renal handling of potassium (Giebisch *et al.*, 1981), reviewed in greater detail in other contributions to this volume, and serves to illustrate the multiple mechanisms by which mineralocorticoid deficien-

TABLE II
SYNDROMES OF HYPOALDOSTERONISM

Primary (hyperreninemic) hypoaldosteronism
Combined mineralcocorticoid and glucocorticoid deficiency
Addison's disease
Bilateral adrenalectomy
Adrenal enzyme deficiency states, 21-hydroxylase deficiency; 3β-ol-dehydrogenase deficiency
Aldosterone deficiency without glucocorticoid deficiency
Corticosterone methyloxidase deficiency, types I and II
Destruction of zona glomerulosa
Acutely ill patients
Heparin therapy
Converting enzyme inhibitors
Secondary (hyporeninemic) hypoaldosteronism
Diabetic nephropathy
Tubulointerstitial diseases
Prostaglandin synthetase inhibitors
Pseudohypoaldosteronism
Type I—endorgan defect (hyperreninemia)
Type II—"chloride shunt" (hyporeninemia)

cy can impair renal potassium handling. This chapter will focus on the pathogenesis and pathophysiological role of mineralocorticoid deficiency (hypoaldosteronism) in abnormalities of potassium homeostasis in humans.

Syndromes of hypoaldosteronism can be divided conveniently into those that result from a primary adrenal abnormality, either a generalized disorder or one localized to the adrenal zona glomerulosa, and those that occur secondary to impaired function of the renin–angiotensin system, the major trophic influence on aldosterone secretion in humans (Table II). Separation of these two pathophysiologically distinct subtypes is achieved readily by measurement of plasma renin activity (PRA), which is usually markedly elevated in the former and reduced in the latter. Hyperkalemia of renal origin that fails to respond to superphysiological doses of mineralocorticoid is called pseudohypoaldosteronism. Two distinct subtypes of pseudohypoaldosteronism have been recognized, and these two also can be readily separated by measurement of PRA.

I. PRIMARY HYPOALDOSTERONISM

A. Generalized Adrenocortical Insufficiency

Generalized adrenocortical insufficiency (Addison's disease) occurs when destruction of adrenocortical tissue reduces secretory rates of both mineralocorticoid and glucocorticoid hormone below the physiological needs of the organism. This combined deficiency is usually fatal if untreated. A similar combined hormonal deficiency results from surgical removal of both adrenal glands and from certain inherited disorders of steroid biosynthesis.

Glucocorticoid hormone deficiency results in anorexia, weight loss, weakness, apathy, and a general inability to withstand "stress." Glucocorticoids normally inhibit ACTH secretion so that markedly elevated levels of ACTH are characteristic of Addison's disease. Hyperpigmentation results from the increased levels of ACTH or related proopiomelanocortin-derived peptides (lipotropin, melanocortin). Mineralocorticoid deficiency results in impaired renal sodium conservation as well as impaired potassium and H^+ secretion in the distal nephron. If the sodium intake is sufficiently large, extracellular fluid volume, plasma potassium, and bicarbonate levels can be maintained at normal or near normal levels. If the sodium intake is low, however, or if extrarenal losses of sodium occur, the inability to conserve sodium maximally results in marked sodium deficits, hyponatremia, hyperkalemia, acidosis, hypovolemia, and increased plasma renin levels. Glucocorticoid deficiency may add to the severity of the hypovolemia by redistributing fluid between vascular and extravascular compartments and to the hyponatremia by impairing renal diluting ability.

Studies in patients with adrenal insufficiency provide evidence that normal circulating levels of aldosterone influence renal clearance of potassium in sub-

jects ingesting moderate amounts of dietary potassium, sodium, and chloride. We examined the role of normal circulating levels of mineralocorticoid in studies performed in adrenalectomized patients maintained on physiological replacement doses of glucocorticoid (dexamethasone) and mineralocorticoid (fludrocortisone) steroids (Sebastian *et al.*, 1980). At least in some such patients, when administration of the mineralocorticoid is selectively discontinued, renal potassium excretion decreases and plasma potassium increases and is sustained at higher levels (Fig. 1). Similarly, in some patients with Addison's disease maintained on constant amounts of hydrocortisone or cortisone, nontreatment with fludrocortisone (or similar potent mineralocorticoid) is associated with a sustained increase in plasma potassium concentration to values greater than those of normal subjects ingesting similar amounts of dietary potassium and sodium (Smith *et al.*, 1984; Thompson, 1979) and greater than those in the same patients treated with physiological replacement doses of fludrocortisone (Thompson, 1979). These data suggest that the level of circulating aldosterone is an important determinant of the set point of renal regulation of plasma potassium concentration and body potassium content in humans ingesting moderate to high levels of dietary potassium.

In patients with Addison's disease, treatment with hydrocortisone or an equivalent glucocorticoid is required on a lifelong basis. Mineralocorticoid replacement is added frequently, but many patients may be maintained on a high sodium intake without mineralocorticoid. The need for mineralocorticoid replacement therapy can be assessed by measurement of PRA, a sensitive index of extracellular volume in patients with Addison's disease. The usual mineralocorticoid steroid administered therapeutically is fludrocortisone, which is effective orally. Although 50–150 μg fludrocortisone per day is the usual recommended dose range, measurements of PRA in patients so treated suggest that such doses may be suboptimal (Smith *et al.*, 1984).

B. 21-Hydroxylase Deficiency

Combined glucocorticoid and mineralocorticoid deficiency also occurs in several congenital disorders of the adrenal gland characterized pathologically by diffuse bilateral enlargement of the adrenal cortex and collectively referred to as congenital adrenal hyperplasia. These disorders result from inherited defects in adrenal steroid biosynthesis inferred to result from adrenal enzyme defects. The reduced secretion of steroids by the adrenal gland leads to supernormal circulating levels of adrenotropic hormones (ACTH and/or angiotensin), which attempt to increase the rate of secretion of the steroids by the defective gland and thereby induce adrenal hyperplasia (Horner *et al.*, 1979). The commonest form of congenital adrenal hyperplasia results from defective 21-hydroxylation in the zona fasciculata and in some, but possibly not all, patients, also in the zona glomeru-

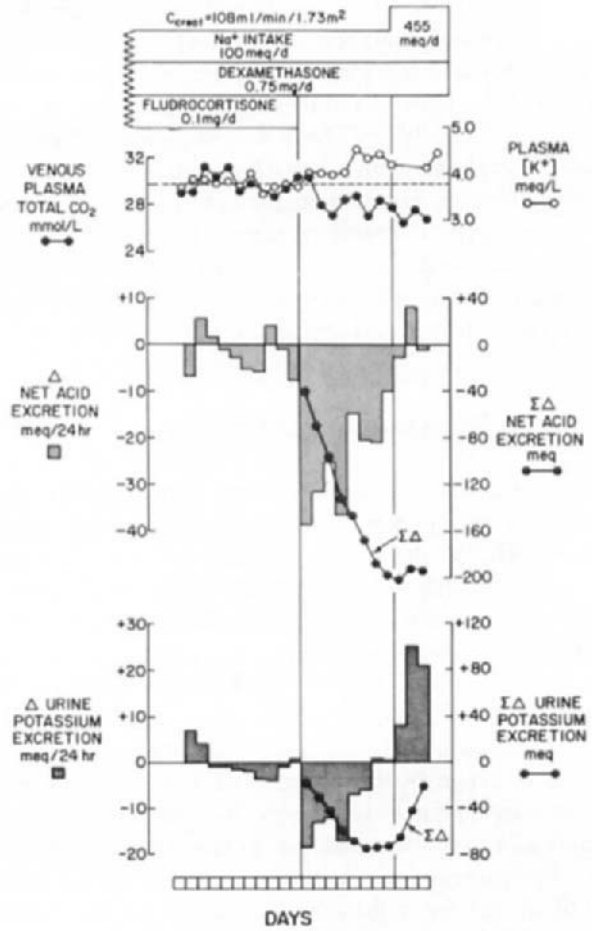

FIG. 1. Effect of selective discontinuation of mineralocorticoid replacement therapy in an adrenalectomized patient who had normal kidneys and who was maintained on a normal intake of sodium chloride. Δ urine potassium and net acid excretion (shaded bars) reflect the differences between the daily excretion rate and the mean excretion rate in the control period (scale at left-hand side of figure). ΣΔ urine potassium and net acid excretion (filled circles) reflect the accumulated daily differences (scale at right-hand side of figure). After mineralocorticoid therapy was discontinued, urine potassium and net acid excretion decreased promptly, remained decreased for 4–6 days, and then returned to control levels. Concomitantly, plasma potassium concentration increased and was sustained at a higher level throughout the period of mineralocorticoid discontinuation. Thus, the set point at which the kidney regulated plasma potassium concentration was higher in the absence of mineralocorticoid. Note that the set point at which the kidney regulated plasma bicarbonate concentration was reduced in the absence of mineralocorticoid therapy. [Reproduced from Sebastian *et al.* (1980) with permission.]

losa. Secretion by the zona fasciculata of 21-hydroxylated steroids is subnormal, whereas secretion of progesterone and 17-hydroxyprogesterone (the normal substrates for 21-hydroxylation) is increased. Signs and symptoms of combined glucocorticoid and mineralocorticoid deficiency are commonly, but not invariably present (Keenan *et al.*, 1979). The increased levels of 17-hydroxyprogesterone in the fasciculata cells result in overproduction of adrenal androgens, causing clinical virilization (female pseudohermaphroditism, male precocious puberty), differing greatly in severity among patients. The increased levels of 17-hydroxyprogesterone and progesterone in the peripheral circulation contribute to the clinical manifestations of mineralocorticoid insufficiency inasmuch as these steroids are mineralocorticoid receptor antagonists that inhibit the renal action of aldosterone.

C. Corticosterone Methyloxidase Deficiency

A deficiency of the mixed function oxidase (corticosterone methyloxidase) required for the final steps of aldosterone biosynthesis results in a syndrome of isolated mineralocorticoid deficiency characterized by salt wasting, hyperkalemia, and metabolic acidosis (Ulick, 1976; Rosler *et al.*, 1977; Veldhuis *et al.*, 1980). The terms corticosterone methyloxidase types 1 and 2 have been proposed to describe the aldosterone deficiency states caused by impaired hydroxylation of corticosterone to 18-hydroxycorticosterone (18-OHB) and of impaired dehydrogenation of 18-OHB to aldosterone, respectively (Ulick, 1976). In patients with the type 2 defect, plasma levels of aldosterone may occasionally be in the "normal" range (Rosler *et al.*, 1977), but these values should be considered to be inappropriately low for the marked degree of hyperreninemia and hyperkalemia that are present and that, in fact, reflect the mineralocorticoid deficient state. The presence of the type 2 defect can be established by the demonstration of an increase in the excretory rate of the major urinary metabolites of 18-OHB (Ulick, 1976). Because corticosterone methyloxidase is not required for the biosynthesis of cortisol, there are no associated abnormalities in the levels of cortisol, ACTH, or adrenal androgen. The severity of the consequences of mineralocorticoid deficiency in affected children can be minimized by the maintenance of a high salt intake or by mineralocorticoid replacement therapy. Whereas the requirements for mineralocorticoid and/or a high salt intake may decrease with age, the impaired ability to conserve sodium in response to a low salt intake persists.

II. SECONDARY HYPOALDOSTERONISM

Other than rare cases of isolated hypoaldosteronism due to corticosterone methyloxidase deficiency, aldosterone deficiency occurring in the absence of

glucocorticoid deficiency is most commonly the result of deficient secretion of renin by the renal juxtaglomerular apparatus, usually associated with diffuse histologically evident renal parenchymal diseases that impair glomerular filtration and renal tubule function (Hudson *et al.*, 1957; Schambelan *et al.*, 1972; Weidmann *et al.*, 1973; Schambelan *et al.*, 1980; DeFronzo, 1980). In these disorders, the proximate cause of aldosterone deficiency is presumed to be a subnormal circulating level of the major hormonal regulator of aldosterone secretion, angiotensin II, the intravascular generation of which from its precursor, angiotensin I, is initiated by renin-mediated enzymatic cleavage of the circulating angiotensin precursor, angiotensinogen, produced in the liver.

In addition to deficient renin secretion, hypoaldosteronism caused by subnormal circulating levels of angiotensin II can occur as a result of drug-induced inhibition of the normal intravascular generation of angiotensin II from angiotensin I (administration of converting enzyme inhibitors) (Warren and O'Connor, 1980) and as a result of impaired hepatic production of angiotensinogen (Landier *et al.*, 1984). A syndrome of hypoaldosteronism possibly due to acquired insensitivity of the adrenal zona glomerulosa to angiotensin II has also been described (Morimoto *et al.*, 1979).

Hyporeninemic Hypoaldosteronism

In adults, mineralocorticoid deficiency occurs in numerous renal and extrarenal disorders that cause diminished renal secretion of renin (Schambelan *et al.*, 1980; DeFronzo, 1980). In affected patients, mineralocorticoid deficiency is manifested as hyperkalemia characteristically and hyperchloremic metabolic acidosis commonly. Sodium depletion and renal sodium wasting are not invariably present (Oh *et al.*, 1974), however, and in some patients, total body sodium and extracellular fluid volume are supernormal, raising the possibility that in such patients deficient renin secretion is a functional consequence of reduced renal clearance of sodium chloride.

The prevalence of aldosterone deficiency in hyperkalemic patients with diffuse renal parenchymal disease and chronic renal insufficiency was investigated in 31 such patients who had creatinine clearances ranging from 10 to 56 ml/min/1.73 m^2 and serum potassium concentrations ranging from 6.0 to 8.7 mEq/liter when dietary potassium was unrestricted at the time of initial diagnosis (Schambelan *et al.*, 1980). None of the patients had cortisol deficiency. By comparison with normal subjects, 14 of the 31 patients had frank hypoaldosteronism. Furthermore, when the effects of concomitant hyperkalemia on aldosterone production were assessed by comparing the ratio of urine aldosterone excretion to the serum potassium concentration, 23 of the 31 patients were judged to have hypoaldosteronism (Fig. 2). Thus, hypoaldosteronism occurs commonly in patients with chronic renal insufficiency who have hyperkalemia.

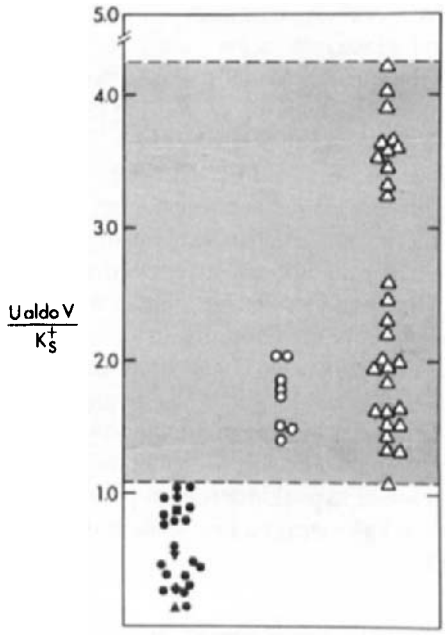

FIG. 2. Values for the ratio of urinary excretion of aldosterone-18-glucoronide to serum potassium concentration ($U_{aldo}V/K_S^+$) in patients with hyperkalemia and chronic renal insufficiency (filled and unfilled circles) and in normal subjects (triangles). Two subgroups of patients are identified: 23 patients (subgroup A, filled circles) in whom values of this ratio are below the range in normal subjects (shaded area) and 8 patients (subgroup B, open circles) in whom the value of this ratio fell within the normal range. [Reproduced from Schambelan *et al.* (1980) with permission.]

The results of our study also indicated that aldosterone deficiency contributed to the pathogenesis of hyperkalemia in these patients. A significantly greater degree of hyperkalemia was observed in the patients with hypoaldosteronism than in those without overt hypoaldosteronism in relation to the net dietary load of potassium (Fig. 3). It is conceivable that the presence of hyperkalemia in the second subgroup, albeit of lesser severity than in those with overt hypoaldosteronism, was related to a lesser degree of hypoaldosteronism in these patients. Viewed in another way, these data indicate that renal clearance of potassium is impaired in hyperkalemic patients with chronic renal insufficiency, and that the magnitude of this impairment is related to the extent to which aldosterone secretion is subnormal.

Hyporeninemia was present in more than 80% of our patients with isolated hypoaldosteronism and appeared to be quantitatively sufficient to account for the degree of aldosterone deficiency (Schambelan *et al.*, 1980). The pathogenesis of hyporeninemia in such individuals is probably multifactorial, including such possibilities as structural damage to the juxtaglomerular apparatus (Sparagana,

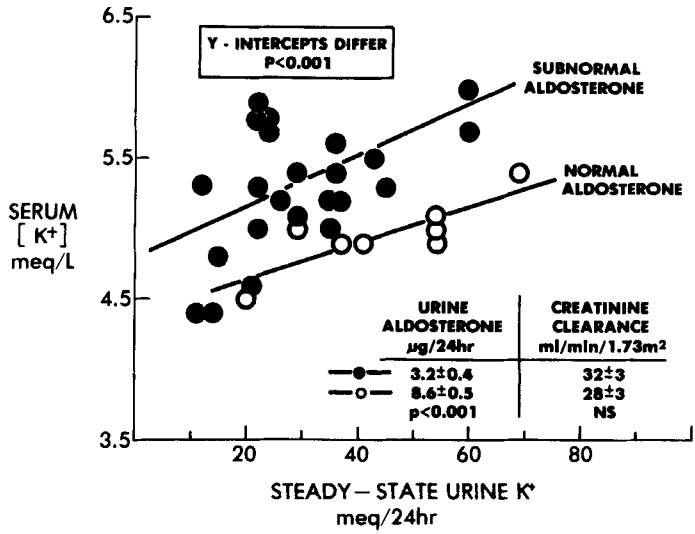

FIG. 3. Relationship between serum potassium concentration and steady-state urinary potassium excretion in patients with isolated hypoaldosteronism (subgroup A, filled circles) and patients with chronic renal insufficiency without hypoaldosteronism (subgroup B, unfilled circles). The findings may be interpreted as reflecting the relationship between serum potassium concentration and dietary potassium intake inasmuch as in the steady state, urinary potassium excretion reflects dietary potassium intake. As indicated by regression lines, a significant positive correlation between the variables was observed both in the patients in subgroup A ($y = 0.02x + 4.72$; $r = 0.56$; $P < 0.01$) and in those in subgroup B ($y = 0.013x + 4.38$; $r = 0.03$; $P < 0.01$). The y intercept was significantly greater ($P < 0.001$) in the patients in subgroup A than it was in those in subgroup B, but the slopes did not differ significantly in the two groups (analysis of covariance). [Replotted from data in Fig. 7 in Schambelan *et al.* (1980) with permission.]

1975), autonomic neuropathy (Tuck *et al.*, 1979), and failure to convert the inactive renin precursor (prorenin) to the active form of the molecule (Luetscher *et al.*, 1985). In some patients, particularly those with diabetes mellitus, it has been suggested that a defect in the final step of aldosterone biosynthesis in the zona glomerulosa may also be present (Tuck and Mayes, 1980).

To study the function of the zona glomerulosa in such patients, we measured levels of plasma aldosterone and its immediate biosynthetic precursor, 18-OHB, in patients with isolated hypoaldosteronism in comparison to those with a variety of adrenal and extraadrenal disorders (Kater *et al.*, 1985). A disproportionate increase in 18-OHB relative to aldosterone was not observed in the group with isolated hypoaldosteronism (Table III). Because concomitant hyporeninemia may have masked the expression of a biosynthetic block, we infused des-Asp^1-angiotensin II (angiotensin III) as a specific stimulus of zona glomerulosa production (Schambelan *et al.*, 1982). In the group with isolated hypoaldosteronism, the response of both aldosterone and 18-OHB to angiotensin III was reduced

TABLE III

EVALUATION OF CORTICOSTERONE METHYLOXIDASE ACTIVITY AS REFLECTED BY THE 18-OHB/ALDOSTERONE RATIO IN DISORDERS AFFECTING THE RENAL–ADRENAL AXIS[a]

Disorder	*N*	Plasma 18-OHB (ng/dl)	Plasma aldosterone (ng/dl)	18-OHB:Aldo ratio	PRC[/PRA] (ng/ml/hr)	Plasma K^+ (mEq/liter)
Aldosterone-producing adenoma	31	163.1 ± 16.3[b]	58.3 ± 6.0[b]	2.8 ± 0.2	0.5 ± 0.1[b]	2.8 ± 0.1[b]
Idiopathic hyperaldosteronism	15	31.4 ± 2.9[c]	15.2 ± 1.2[b]	2.1 ± 0.2	0.9 ± 0.2[b]	3.3 ± 0.1[b]
Salt-losing nephropathy	3	485 ± 208[b]	197 ± 119[b]	3.1 ± 0.5	[6.3 ± 1.9][b]	5.4 ± 0.5[b]
Bartter's syndrome	9	43.5 ± 7.2[d]	15.3 ± 2.7[d]	2.9 ± 0.2	[11.1 ± 1.4]	2.7 ± 0.1[b]
Isolated hypoaldosteronism	13	15.4 ± 2.4[d]	4.9 ± 0.5[b]	3.2 ± 0.4	1.4 ± 0.3[b]	5.2 ± 0.1[b]
Normal control subjects	15	23.3 ± 2.2	8.9 ± 0.5	2.6 ± 0.2	[1.6 ± 0.2] 5.1 ± 0.1	4.1 ± 0.1

[a] Data are abstracted from Table 1 in Kater *et al.* (1985) with permission. Values are mean ±SEM. Comparison between patient groups and normal control subjects was done using Student's *t* test for unpaired variables.

[b] $P < 0.001$.

[c] $P < 0.05$.

[d] $P < 0.01$.

significantly in comparison to normal controls and those with chronic renal insufficiency without hypoaldosteronism. The impaired aldosterone and 18-OHB secretory responses in patients with isolated hypaldosteronism were similar to the response in patients who had recently undergone unilateral adrenalectomy for an aldosterone-producing adenoma; in such patients, the prolonged hypermineralocorticoid state present preoperatively results in suppression of the function of the contralateral adrenal gland and a transient state of hypoaldosteronism postoperatively (Kater and Biglieri, 1982). The impaired response of both 18-OHB and aldosterone to angiotensin III indicates a generalized reduction in zona glomerulosa function rather than an enzymatic defect in adrenal biosynthesis as the cause of hypoaldosteronism in patients with isolated hypoaldosteronism. Such an impairment may result from prolonged hyporeninemia in this syndrome.

Other authors have suggested that mechanisms other than hyporeninemia may account for hypoaldosteronism in some patients with this syndrome, since aldosterone levels remain low despite hyperkalemia, a known and potent stimulus to aldosterone secretion (Dluhy *et al.*, 1972). To evaluate the relative roles of potassium and angiotensin in the control of aldosterone secretion in patients with chronic renal insufficiency, we administered the angiotensin converting enzyme inhibitor, captopril, to patients from the subgroup without hypoaldosteronism (Peters *et al.*, 1983). This resulted in a prompt increase in plasma potassium to levels comparable to those seen in the subgroup with hyporeninemic hypoaldosteronism. Despite the hyperkalemia, urinary aldosterone excretion decreased to levels not significantly different from those in patients with hyporeninemic hypoaldosteronism, presumably as a result of the reduction in angiotensin II levels. Normal subjects studied similarly had no significant change in plasma potassium or urinary aldosterone excretion; presumably the reduction in angiotensin II levels was not as great, owing to the much greater increases in PRA that occurred in the normal subjects. Thus, captopril can unmask an impaired renin secretory response mechanism in patients with chronic renal insufficiency, resulting in hypoaldosteronism, renal potassium retention, and hyperkalemia. Further, these studies provide further support for the concept that angiotensin II is required for a normal adrenal secretory response to potassium (Pratt, 1982).

III. PSEUDOHYPOALDOSTERONISM

Hyperkalemia associated with abnormally low renal clearance of potassium occurs in several clinical disorders referred to by the term pseudohypoaldosteronism because the signs and symptoms suggest that aldosterone deficiency is present, but, in fact, aldosterone levels are normal or supernormal and administration of exogenous mineralocorticoid even in large amounts is not ameliorative.

Two pathophysiologically distinct forms of pseudohypoaldosterone have been described.

A. Type I Pseudohypoaldosteronism

Classic type I pseudohypoaldosteronism is an apparently congenital and, in some cases, a familial disorder characterized by failure to thrive, dehydration, and hyponatremia due to renal salt wasting, hyperkalemia due to renal potassium retention, and renal tubular acidosis (Rosler *et al.*, 1977; Cheek and Perry, 1958; Donnel *et al.*, 1959; Raine and Roy, 1962; Postel-Vinay *et al.*, 1974). In affected infants, PRA and plasma and urinary aldosterone concentrations are markedly elevated, and neither glucocorticoid deficiency nor excess is present. Refractoriness of renal sodium wasting and potassium retention to mineralocorticoid administration is evident even during prolonged periods of parenteral administration of large amounts of deoxycorticosterone and aldosterone and oral administration of fludrocortisone. Supplementation of the diet with large amounts of sodium chloride can greatly ameliorate hyponatremia and hyperkalemia, relieve symptoms, and permit normal or improved growth. Characteristically, the severity of renal sodium wasting and potassium retention diminishes after the period of infancy, permitting discontinuation of sodium chloride supplements without recurrence of overt hyponatremia or hyperkalemia. Disordered renal handling of sodium and potassium persists, however, evidenced by recurrence of hyponatremia and hyperkalemia in response to restriction of dietary sodium chloride (Postel-Vinay *et al.*, 1974).

The pathophysiological manifestations of pseudohypoaldosteronism of infancy are consistent with a cellular defect that interferes with the action of aldosterone in those segments of the renal tubule normally responsive to aldosterone, specifically the collecting tubules and ducts. Generalized glomerular and tubular dysfunction is not present in affected patients, and examination of biopsied renal tissue has usually revealed only the presence of mild hyperplasia of the juxtaglomerular apparatus. These patients are thus distinguishable from patients with type I pseudohypoaldosteronism associated with acquired generalized renal parenchymal destruction (e.g., salt-losing nephritis caused by methicillin nephritis, medullary cystic disease).

Recently, Armanini *et al.* (1985) demonstrated a decreased number of type I mineralocorticoid binding sites in monocytes obtained from three patients with pseudohypoaldosteronism of infancy, including the index case reported by Cheek and Perry (1958). Because the steroid specificity of these receptors is indistinguishable from the renal minerolocorticoid receptor, it seems quite likely that a similar deficiency of type I receptors is present in the kidney. It is not

certain that the same fundamental pathogenetic disturbance underlies classic pseudohypoaldosteronism of infancy in all reported cases, however, nor is it certain that a genetic abnormality is present in all cases. The familial occurrence of the disorder has been documented in only a small fraction of the reported cases.

B. Type II Pseudohypoaldosteronism

Renal tubular responsiveness to the action of aldosterone also appears to be impaired in a rare syndrome characterized by hyperkalemia, hyperchloremic metabolic acidosis, hypertension, hyporeninemia, and abnormally reduced aldosterone production (Arnold and Healy, 1969; Gordon *et al.*, 1970; Brautbar, *et al.*, 1978; Schambelan *et al.*, 1981). Glomerular filtration rate is in the normal range. Mineralocorticoid resistance is apparent by persistence of hyperkalemia and a markedly reduced kaliuretic response to large amounts of exogenously administered mineralocorticoid steroids. The antinatriuretic and antichloruretic responses to mineralocorticoid steroids may be intact. A primary defect in renal potassium secretion has been proposed.

In a patient with type II pseudohypoaldosteronism studied by our group, we found that fractional renal potassium excretion was subnormal and increased only minimally during administration of large amounts of mineralocorticoid steroids (Schambelan *et al.*, 1981). However, distal tubule potassium secretion increased greatly when distal sodium delivery was increased with anions other than chloride (sulfate or bicarbonate) (Fig. 4). Thus, hyperkalemia and mineralocorticoid resistance did not appear to be due to an intrinsic defect in renal potassium secretion. The finding of normal salivary and fecal secretion of potassium further mitigated against a generalized defect in transepithelial transport of potassium. The result of our studies indicate that mineralocorticoid-resistant renal hyperkalemia and acidosis in type II pseudohypoaldosteronism cannot be attributed to the absence of a renal potassium secretory mechanism, but instead may be dependent upon the amount of chloride available for reabsorption in the distal nephron (Schambelan *et al.*, 1981). The findings suggest that the primary abnormality in this syndrome is a defect of the distal nephron that increases the reabsorptive avidity of the distal nephron for chloride, which (1) limits the sodium and mineralocorticoid-dependent voltage driving force for potassium and hydrogen secretion, resulting in hyperkalemia and acidosis, and (2) augments distal sodium chloride reabsorption resulting in hyperchloremia, volume expansion, and hypertension. Such a "chloride shunt" might arise as a result of an abnormal increase in the permeability of the distal nephron to chloride. Consistent with the presence of such a chloride shunt, restriction of dietary sodium

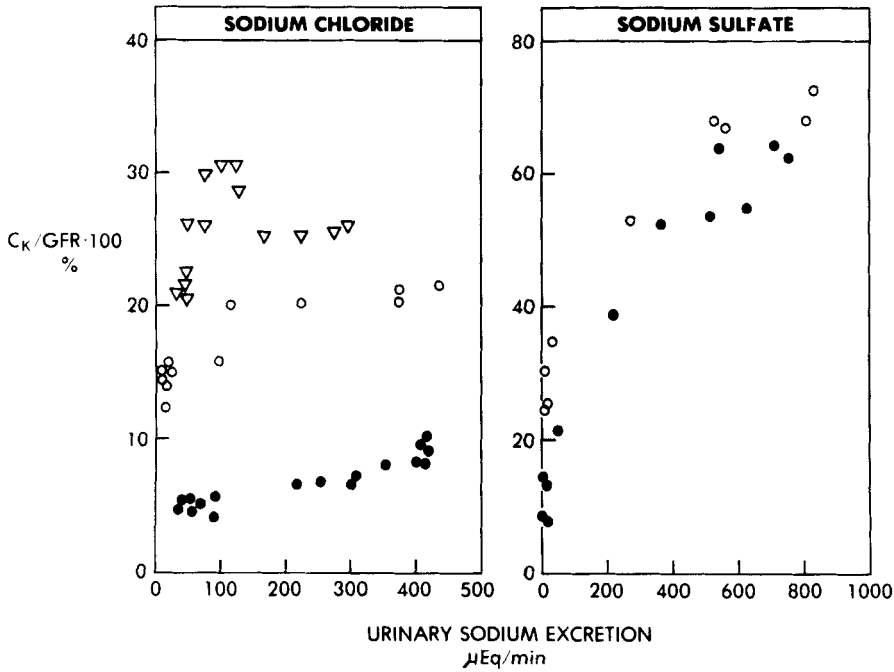

FIG. 4. Relationship between fractional renal potassium clearance (C_K/GFR·100) and urinary sodium excretion during infusion of sodium chloride (left) and sodium sulfate (right) in a patient with type II pseudohypoaldosteronism (filled circles) and normal subjects (unfilled symbols). Mineralocorticoid hormone (DOCA, 10 mg intramuscularly) was given 12 and 2 hr before the study. [Reproduced from Schambelan *et al.* (1981) with permission.]

chloride or administration of a chloruretic diuretic (furosemide, thiazide) ameliorates hyperkalemia and acidosis (Gordon *et al.*, 1970; Lee *et al.*, 1979; Schambelan *et al.*, 1981).

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Chapter 16

Hypokalemia

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I. INTRODUCTION

Clinical disturbances of potassium balance are common and often pose a serious threat to life. Prompt intervention based on sound physiological principles is frequently required to avert catastrophe.

The normal range for serum potassium concentration is ~3.8–5.0 mEq/liter. In a normal individual ingesting a diet of relatively constant potassium content, serum potassium concentration varies by as little as a few tenths of a milliequivalent per liter from day to day. Because such a small fraction of the body's potassium is contained in the extracellular compartment and because transcellular shifts of this cation occur frequently, serum potassium concentration affords only a rough estimate of total body stores. Even when the factors known to cause transcellular potassium shifts are excluded, the relationship between serum potassium concentration and total body potassium is only approximate. As a rough guide, each milliequivalent per liter decrement in serum potassium concentration corresponds to a loss of ~300–400 mEq (Sterns *et al.*, 1981).

II. POTASSIUM DEPLETION AND HYPOKALEMIA

True potassium depletion, defined as a disproportionate reduction in total body potassium with respect to nitrogen balance, is a very common clinical occurrence. Although the clinical circumstance in which one encounters a given patient often serves to heighten the suspicion that potassium depletion exists, it is the finding of hypokalemia that usually establishes its presence. On some occasions, significant potassium deficits coexist with normal or even elevated serum potassium concentration (e.g., diabetic ketoacidosis). Somewhat more com-

monly but still infrequently, significant hypokalemia coexists with normal body potassium stores; examples include acute alkalemia, insulin administration, and the crisis of familial hypokalemic periodic paralysis. Hypokalemia due to transcellular shifts is discussed more fully in Chapter 13.

III. CAUSES OF POTASSIUM DEPLETION

The causes of true potassium depletion can be divided into three broad categories: decreased intake, extrarenal losses, and increased renal excretion.

A. Decreased Intake

The mechanisms for potassium conservation by the kidney are considerably less efficient that those for sodium conservation. Thus, although potassium excretion falls when potassium is removed from the diet, several days are required to achieve minimal rates of excretion and, even then, combined urinary and fecal losses of at least 10–20 mEq/day continue to occur (Welt *et al.*, 1960). Because of the widespread availability of potassium in common foodstuffs, potassium intake rarely falls below these minimum levels of obligatory potassium excretion (Squires and Huth, 1959). Consequently, potassium depletion and hypokalemia rarely occur solely as a result of decreased intake. Moreover, precise quantitation of the true potassium deficit resulting from prolonged dietary deprivation is very difficult to obtain because of the variable contribution made by the concomitant negative nitrogen balance.

B. Extrarenal Potassium Losses

Potassium losses occur quite frequently through the gastrointestinal tract. Occasionally, losses through the skin can result in significant depletion.

Vomiting and gastric drainage almost invariably result in some degree of hypokalemia and potassium depletion. Much of the potassium deficit can be traced to the renal losses induced by the accompanying metabolic alkalosis rather than to the gastrointestinal losses per se. The potassium concentration of gastric fluid is relatively low and fluctuates within a narrow range (~10–20 mEq/liter) irrespective of the level of gastric pH (Witten and Bickel, 1970).

Fecal excretion of potassium normally ranges between 5 and 15 mEq/day. Diarrhea can increase this rate of loss dramatically. The magnitude of the potassium deficit produced by diarrhea may be especially great in patients with prolonged diarrhea even when the daily volume of stool is not especially large. For example, patients who abuse laxatives may develop severe potassium deple-

tion (Schwartz and Relman, 1953). Heavy laxative abuse can also result in sodium depletion, hyperreninemia, and hyperaldosteronism, thus mimicking the findings of Bartter's syndrome. Laxative abuse occasionally stems from an underlying psychiatric disorder and may be associated with anorexia nervosa and/or surreptitious vomiting (Wolff *et al.*, 1968). Villous adenomas of the colon typically secrete large volumes of a mucus-containing fluid that is rich in potassium, and severe hypokalemia may be the result (Babior, 1966).

The potassium concentration in sweat is ~5–10 mEq/liter (Grand *et al.*, 1967). Consequently, large volumes of sweat are required to produce a significant deficit of this cation. Sweat volume in excess of 10 liters per day has been documented to occur during vigorous exercise in hot climates; potassium deficits thus acquired may contribute importantly to the development of rhabdomyolysis in this setting (Knochel *et al.*, 1972).

C. Increased Renal Excretion

Although the rate of potassium excretion in healthy individuals appears to be regulated predominantly by changes in intracellular (i.e., renal epithelial cell) potassium content, several other factors are known to influence potassium excretion importantly and to carry the potential for disrupting potassium balance. These factors, detailed elsewhere in this volume, include the flow rate of fluid in the distal nephron, distal sodium delivery, the nature of the filtered anion load, the circulating level of adrenocortical hormones, and the degree of urinary acidification. Most of the clinical causes of renal potassium loss, which are listed in Table I, can be related to an abnormality in one or more of these factors.

1. Diuretics

The potent diuretics commonly employed (e.g., thiazides, furosemide) result in an immediate increase in urinary potassium excretion. The mechanisms involved in diuretic-induced kaliuresis are reviewed in Chapter 14.

Despite the brisk kaliuresis characteristically observed at the initiation of potent diuretic therapy, large potassium deficits rarely accumulate, even in the presence of continued diuretic treatment. Indeed, in the absence of hyperadrenocorticism (primary or secondary) or other independent kaliuretic influences, potassium deficits induced by diuretics do not continue to worsen after the first week or so of therapy (Morgan and Davidson, 1980). There is evidence that thiazide diuretics produce a somewhat larger mean reduction in serum potassium concentration than does furosemide, but in neither case does serum potassium typically fall by more than several tenths of a milliequivalent per liter; at the usual doses employed in the treatment of hypertension, the average reduction in serum potassium concentration produced by thiazides is ~0.6 mEq/liter whereas

TABLE I
HYPOKALEMIA DUE TO INCREASED RENAL EXCRETION

Diuretics
Thiazides
"Loop" agents
Osmotic
Carbonic anhydrase inhibitors
Acid–base disturbances
Tubular diseases
Distal RTA
Proximal RTA (especially with Fanconi syndrome)
Bartter's syndrome
Recovery from ATN (?)
Recovery from obstruction (?)
Hyperadrenocorticism
Primary aldosteronism
Cushing's syndrome (especially ectopic ACTH)
Licorice ingestion
Certain forms of hyperreninemia
Magnesium deficiency

that produced by furosemide is ~0.3 mEq/liter (Morgan and Davidson, 1980). The magnitude of the associated deficit of body potassium averages ~200–300 mEq at most. Indeed, currently available methods for estimating total body potassium often fail to detect any reduction in body potassium stores. Only 5% or less of patients treated with these agents experience a fall in serum potassium concentration to <3.0 mEq/liter.

Diuretic-induced potassium losses are fostered by the ingestion of diets high in sodium, presumably because of the increased sodium traffic through the distal nephron where potassium secretory sites prevail. Paradoxically, diuretic-induced potassium losses are also fostered by the ingestion of diets severely restricted in sodium; the increased kaliuresis observed under these circumstances probably reflects the increased levels of aldosterone characteristic of the sodium-deprived state and the concurrent presence of metabolic alkalosis and hypochloremia.

Osmotic diuresis due to hyperglycemia or to the administration of mannitol is typically associated with an increase in urinary excretion of potassium. In diabetics, as much as 150 mEq of potassium can be lost within 1 day following discontinuation of insulin therapy, even in the absence of ketoacidosis (Atchley *et al.*, 1933). The ordinary doses of mannitol employed in promoting an osmotic diuresis are associated with less severe urinary potassium losses; 10–20 mEq of potassium are typically excreted within the first 3 hr of a mannitol diuresis (Gennari and Kassirer, 1974).

Carbonic anhydrase inhibitors are rarely used any longer solely for their diuretic properties, but they do find useful application in the treatment of glaucoma, in the prevention of mountain sickness, and in circumstances in which rapid alkalinization of urine is desired. These agents produce an alkaline diuresis by inhibiting proximal tubule carbonic anhydrase. The resulting distal delivery of bicarbonate is held responsible for the marked increase in potassium excretion that often accompanies use of this agent.

2. Acid–Base Disturbances

a. General Features. Acute acid–base disturbances have well-known effects on renal potassium excretion. An acute decrease in systemic pH, due either to respiratory or metabolic acidosis, results in an immediate reduction in urinary potassium excretion. This antikaliuretic effect is presumably mediated by the acidosis-induced shift of potassium out of distal tubular cells. Conversely, an acute increase in systemic pH and a shift of potassium into cells results in an immediate increase in potassium excretion.

These acute, pH-mediated effects, however, are of relatively short duration and give way to other more potent influences if the acid–base disturbance persists for more than a few hours (Gennari and Cohen, 1975). During the first few days of experimental chronic metabolic acidosis, for example, renal potassium excretion increases markedly; as a result, hypokalemia and total body potassium depletion of considerable magnitude develop. Because this striking kaliuresis occurs in the presence of persistent acidemia, it is clear that the acute inhibitory effect of reduced pH on distal tubular potassium secretion is overridden by other factors. Enhanced distal sodium delivery and distal tubular sodium reabsorption is hypothesized to explain the kaliuresis characteristics of this acid–base disorder (DeSousa *et al.*, 1974). As with metabolic acidosis, the immediate reduction in potassium excretion observed at the onset of respiratory acidosis is followed shortly thereafter by an increase in potassium excretion. As a result, a mild to moderate potassium deficit accumulates as early as 24 hr later. The magnitude of the potassium deficit acquired during experimental chronic respiratory acidosis is usually less than that acquired during chronic metabolic acidosis (Gennari and Cohen, 1975).

The immediate increase in potassium excretion observed during acute respiratory alkalosis is not a feature of more sustained hypocapnia. Indeed, animals with experimental chronic respiratory alkalosis manifest no significant change in potassium balance if the dietary intake of sodium chloride is normal (Gennari *et al.*, 1972). In contrast, chronic metabolic alkalosis is virtually always associated with significant potassium depletion. This uniform association once led to the speculation that potassium depletion was responsible for the elevated renal bicarbonate reabsorption characteristic of chronic metabolic alkalosis. The weight of

evidence, however, now strongly favors the veiw that potassium deficits observed with the common forms of metabolic alkalosis are the consequence rather than the cause of the acid–base disorder (Schwartz *et al.*, 1968).

b. Alkali Loading. The intravenous administration of alkali typically results in a fall in serum potassium concentration. This effect is due in part to a shift of potassium into cells and in part to an increase in potassium excretion. Oral administration of alkali can also result in hypokalemia. The hypokalemia induced by alkali loading is rarely severe; in one study, serum potassium never fell below 3 mEq/liter (van Goidsenhoven *et al.*, 1954).

c. Diabetic Ketoacidosis. Patients with diabetic ketoacidosis typically experience significant renal loss of potassium before reaching medical attention. At least two major mechanisms are responsible: (1) the osmotic diuresis induced by intense glycosuria, and (2) the increased excretion of nonreabsorbable ketoacid anions. The renal potassium losses incurred by patients with diabetic ketoacidosis may be compounded by gastrointestinal losses due to the vomiting that often accompanies uncontrolled diabetes.

Despite the presence of significant potassium depletion in virtually all patients with diabetic ketoacidosis, only a small percentage manifest hypokalemia when first evaluated (Beigelman, 1971). Indeed, the internal redistribution of potassium responsible for this paradox may be so pronounced that frank hyperkalemia may be present before treatment, even when sizable potassium deficits exist.

3. Tubular Diseases

a. Renal Tubular Acidosis. Hypokalemia and potassium depletion are characteristic features of distal ("gradient limited," Type I) RTA and of those instances of proximal ("bicarbonate wasting," Type II) RTA accompanied by the Fanconi syndrome (Gennari and Cohen, 1978; Milne *et al.*, 1952). In patients with distal RTA, correction of systemic acidosis by alkali therapy usually results in the restoration of normal serum potassium concentration (Sebastian *et al.*, 1971a). Alkali therapy also tends to repair the extracellular volume deficits seen in patients with distal RTA, thus raising the possibility that the reduction in potassium excretion is secondary to a dampening of aldosterone secretion. Administration of sodium chloride, however, results in similar reductions in aldosterone secretion, but in no reduction in potassium excretion (Gill *et al.*, 1967). In some patients with distal RTA, potassium balance cannot be restored unless correction of the acidosis is coupled with the provision in daily potassium supplements (Sebastian *et al.*, 1971b).

Patients with isolated proximal RTA can apparently maintain normal or near

normal potassium balance. Those with associated features of the Fanconi syndrome typically manifest some degree of hypokalemia. In both groups, alkali therapy is accompanied by an increase in potassium excretion and a fall in serum potassium concentration (Sebastian *et al.*, 1971a). In patients with some degree of potassium depletion prior to initiation of therapy, additional alkali-induced losses of potassium can lower serum potassium concentration to potentially dangerous levels (Sebastian *et al.*, 1971b). The kaliuretic effect of alkali in patients with proximal RTA can be explained as follows: Alkali loading in the presence of a diminished capacity for proximal bicarbonate reabsorption increases distal delivery of bicarbonate (and sodium), thereby enhancing potassium secretion. Long-term diuretic therapy, which is often employed in patients with proximal RTA to enhance proximal bicarbonate reabsorption, may greatly aggravate the potassium-wasting tendency (Donckerwolcke *et al.*, 1970).

b. Bartter's Syndrome. Bartter's syndrome is a very rare condition, but one in which hypokalemia and potassium depletion occur sine qua non. Renin and aldosterone secretion are elevated in this syndrome. Patients often present with asymptomatic hypokalemia, but weakness due to potassium depletion is well described (Bartter *et al.*, 1962).

4. Hyperadrenocorticism

Virtually all forms of hyperadrenocorticism are characterized by enhanced potassium excretion and some degree of hypokalemia. This kaliuretic tendency reflects, in large part, the ability of mineralocorticoids to stimulate distal tubule potassium secretion when ample sodium is being ingested and excreted. In addition, hyperadrenocorticism is often associated with increased catabolism due to glucocorticoid effects; thus, a substantial portion of the renal potassium losses seen in patients with Cushing's syndrome and in patients receiving large therapeutic doses of steroids is accounted for by negative nitrogen balance and does not contribute to true potassium depletion.

a. Primary Aldosteronism. In patients with primary aldosteronism, the degree of hypokalemia and potassium depletion is highly variable. The degree of potassium depletion is influenced importantly by the level of sodium intake. This finding is in keeping with experimental observations that mineralocorticoid administration to subjects or animals ingesting salt-free diets does not alter potassium (or acid–base) balance (Relman and Schwartz, 1952). The kaliuretic effect of dietary sodium is due to the added stimulus to potassium secretion provided by increased delivery of sodium to the distal nephron. The ability to sodium loads to accelerate renal potassium excretion is so characteristic of primary aldosteronism that it has been used to help establish the diagnosis of this

condition (George *et al.*, 1970). An electrolyte disturbance similar to that seen in primary aldosteronism can be produced by the ingestion of large quantities of licorice, which contains glycyrrhizic acid, a plant steroid with strong mineralocorticoid activity (Conn *et al.*, 1968).

b. Cushing's Syndrome. In patients with Cushing's syndrome, the degree of hypokalemia varies considerably and correlates reasonably well with the circulating levels of cortisol. This correlation may be fortuitous in view of the likelihood that the hypokalemia in this setting is actually a consequence of increased secretion of deoxycorticosterone B; aldosterone itself has not been implicated (Kaplan, 1974). Hypokalemia is much more frequent and more severe in patients with ectopic ACTH production than in those with either pituitary or adrenal forms of Cushing's syndrome (Allot and Skelton, 1960).

c. Hyperreninemic States. The common edema-forming states (i.e., congestive heart failure, cirrhosis, nephrotic syndrome) are frequently associated with hyperreninemia and with hypokalemia and potassium depletion. Loss of potassium has often been assumed to be a consequence solely of the increased aldosterone secretion ("secondary aldosteronism") characteristic of these conditions. However, hypokalemia does not occur in experimental edema-forming states in which aldosterone secretion is markedly elevated (Levy, 1972). Moreover, edematous patients with secondary aldosteronism do not become hypokalemic or increase urinary potassium excretion even when sodium loading assures adequate distal delivery. Therefore, when significant potassium depletion develops in the presence of sodium retention, it is likely that kaliuretic factors other than aldosteronism per se are at play. Such factors as diuretic administration and metabolic alkalosis are so frequently superimposed on these disease states that potassium depletion is in fact often clinically manifest. Hypokalemia has also been described in other forms of secondary aldosteronism and hyperreninemia (e.g., malignant hypertension, renal artery stenosis).

5. Magnesium Deficiency

Magnesium deficiency has been reported in association with hypokalemia and potassium depletion in a wide variety of diseases including intestinal malabsorption, delirium tremens, primary hyperparathyroidism and primary aldosteronism, as well as with long-term gentamycin therapy. It is not clear, however, whether the association between magnesium and potassium deficiency stems from a cause and effect relationship or from a coincidental loss of both cations due to the underlying abnormality. That magnesium deficiency may cause renal potassium wasting is suggested by the kaliuresis that results from experimental magnesium depletion (Shils, 1969). Other circumstances in which both magnesium and

potassium deficits may coexist include cis-platinum administration, diuretic administration, and diabetic ketoacidosis.

6. Miscellaneous Causes of Renal Potassium Wasting

The administration of amphotericin B frequently results in hypokalemia due to renal potassium wasting (McCurdy *et al.*, 1968). Serum potassium levels as low as 1.0 mEq/liter have been reported in patients receiving large doses of this potent artifungal agent (McChesney and Marquardt, 1964). Carbenicillin administered in large doses may cause increased potassium excretion and severe hypokalemia. Renal potassium wasting has also been described, albeit less frequently, in association with other antibiotics, including ampicillin, penicillin, polymixin B, and gentamycin.

Hypokalemia due to renal potassium wasting occurs in a sizable proportion of patients with monocytic or myelomonocytic leukemia (Muggia *et al.*, 1969). On occasion, the potassium wasting is associated with evidence of Fanconi's syndrome. Speculation about the cause of potassium wasting in this setting has centered about the role of lysozymuria, which is also frequently seen in these patients. Lysozymuria, however, is also seen in the absence of potassium wasting.

IV. ADVERSE CONSEQUENCES OF HYPOKALEMIA AND POTASSIUM DEFICITS

Potassium deficits are known to produce a large number of potentially adverse effects which can be subclassified according to Table II.

A. Renal Effects

Moderate potassium deficits tend to reduce GFR and to reduce the maximum concentrating capacity of the kidney (Rubini, 1961). The latter defect cannot be reversed by the administration of antidiuretic hormone, but rarely does the severity of the concentrating defect pose a significant clinical problem. Both GFR and concentrating capacity are restored to normal when potassium stores are repleted.

Potassium depletion fosters renal ammoniagenesis, a change that has been hypothesized to facilitate renal potassium conservation by enabling the kidney to shift obligatory distal sodium reabsorption toward sodium–hydrogen and away from sodium–potassium exchange (Tannen, 1970). In patients with a propensity for developing hepatic encephalopathy, diuretic-induced potassium losses and the resulting augmentation of renal ammoniagenesis may be hazardous.

Potassium deficits enhance renal sodium conservation and, hence, may foster

TABLE II
ADVERSE CONSEQUENCES OF HYPOKALEMIA

Disturbance in renal function
↓ GFR
↓ Concentrating ability
↑ Na reabsorption
↑ Ammoniagenesis
Disturbances in cardiovascular function
Potentiates digitalis-induced arrhythmias
↑ Ventricular premature contractions (?)
↑ Risk of VT and VF during acute MI (?)
Potentiates hypertension
Disturbances in metabolism
Glucose intolerance
Lipid abnormalities (?)
Disturbances in muscle function
Skeletal muscle weakness
↓ Deep tendon reflexes
Respiratory paralysis
Adynamic ileus
Rhabdomyolysis
Other (?)
Fatigue, lassitude
Cramps

the development of edema and hypertension. Other mechanisms may also exist by which potassium depletion influences blood pressure control.

B. Cardiovascular Effects

The most worrisome of the potential adverse effects of potassium depletion involve cardiac conduction and rhythm. The theoretical basis for concern is the well-established effect of hypokalemia on resting membrane potential and, more importantly, on the repolarization phase of the cardiac action potential. Hypokalemia results in hyperpolarization and, following depolarization, in a progressive delay in repolarization. The cardiac conduction system is more severely affected by a given degree of hypokalemia than is ventricular muscle. Delayed repolarization lengthens the interval during which the membrane potential is near the threshold level and thus increases the risk of automaticity and reentry events. Increased ventricular ectopy and arrhythmias might be anticipated on this basis.

At the clinical level, the potentiating effects of hypokalemia on digitalis-induced arrhythmias are beyond doubt. It is much less clear, however, whether

the electrophysiological effects of mild hypokalemia (e.g., of the magnitude typically produced by diuretics) are manifest as increased ventricular ectopy in patients with normal hearts or even in those with diseased hearts. Several studies of hypertensive patients treated with diuretics have been unable to document increased ectopy (Papademetriou *et al.*, 1983; Madias *et al.*, 1984; Lief *et al.*, 1983) whereas others appear to have done so (Holland *et al.*, 1981; Hollifield, 1984; Caralis *et al.*, 1984; Medical Research Council Working Party on Mild to Moderate Hypertension, 1983). Even when ectopy has been observed, however, the causal role of hypokalemia can be questioned because of the frequency with which confounding factors are also present (Harrington *et al.*, 1982). Based on the available data, a reasonable suspicion can be raised, at least in patients with underlying cardiac disease, that diuretic-induced hypokalemia is associated with some increase in ventricular ectopy. If so, the clinical significance of the increased ectopy is far from established.

Several studies have documented the increased likelihood of patients with myocardial infarction (MI) developing ventricular tachycardia or ventricular fibrillation if hypokalemia is also present (Solomon and Cole, 1981; Nordrehaug, 1981; Hulting, 1981; Dyckner *et al.*, 1982; Reuben and Kun, 1983; Johansson and Dziamski, 1984). Indeed, patients with progressively more severe degrees of hypokalemia appear to have a progressively greater chance that serious ventricular arrhythmias will develop in the early stages of recovery from an acute MI. Once again, however, a causal role for hypokalemia has not been established. It is possible that hypokalemia in the setting of an acute MI is merely a marker for the presence of other arrhythmogenic factors (e.g., increased catecholamines). Hypokalemia is known to result from increased catecholamines (Struthers *et al.*, 1983). The results of prophylactic treatment of hypokalemia in an effort to forestall the development of ventricular tachycardia and ventricular fibrillation in patients with acute myocardial infarction have not been reported.

The presence of diuretic-induced hypokalemia in hypertensive patients is associated with a modest reduction in blood pressure control; potassium replacement therapy results in an average reduction in mean arterial pressure of ~5 mm Hg (Kaplan *et al.*, 1985).

C. Muscle Metabolism

The adverse effects of potassium depletion on muscle metabolism are reviewed extensively in Chapters 17 and 18. Clinical manifestations include weakness, respiratory muscle paralysis, adynamic ileus, and, often in conjunction with other factors, rhabdomyolysis.

V. REPAIR OF POTASSIUM DEFICITS

A detailed discussion of the clinical therapeutics of potassium replacement is beyond the scope of this chapter. Suffice it to say that sizable potassium deficits (e.g., greater than 400–500 mEq; serum potassium concentration <3.0 mEq/liter) should invariably be repaired despite the risk associated with replacement therapy. When the magnitude of the deficit is more modest (less than 300 mEq; serum potassium concentration 3.1–3.5 mEq/liter), a judgment must be made as to whether the benefits of replacement outweigh the risks. Similarly, a judgment must be made regarding the prophylactic use of potassium supplements or potassium-sparing agents in patients treated with conventional diuretics for hypertension or edema; as noted above, in the absence of prophylactic potassium maintenance, the vast majority of such patients develop only modest degrees of potassium depletion as evidenced by serum potassium levels that remain above 3.0 mEq/liter, indeed often well above.

The most reliable means for repairing established deficits of potassium is to administer potassium as the chloride salt. Potassium chloride can be given parenterally if necessary but, whenever possible, the oral route should be employed. When attempting to prevent the development of potassium deficits (e.g., in patients receiving diuretics, see later), oral KCl supplements are far more efficacious than oral potassium supplements administered with any other anion; renal retention of administered potassium is greatly facilitated when chloride is abundantly available. As a rule, 40–80 mEq/day of KCl is sufficient to obviate development of diuretic-induced hypokalemia.

As an alternative to KCl supplements in preventing potassium depletion, one may administer an agent that inhibits renal potassium secretion (i.e., triampterin, amiloride, or spironolactone). In appropriate dosage, such agents are at least as efficacious as KCl (Morgan and Davidson, 1980).

The principal danger both of potassium supplements and of potassium-sparing agents is, of course, the development of life-threatening hyperkalemia. Unfortunately, adequate data are not available to assess fully the risks entailed in potassium replacement. One multicenter, retrospective study of inpatients estimated the risk of developing life-threatening hyperkalemia to be ~1 in 200 patients treated either with KCl or with potassium-sparing agents (Lawson, 1974). The risk is greater when potassium salts and potassium-sparing agents are administered concomitantly. The risk is also greater in patients with renal insufficiency, in those with diabetes, and in the elderly. Certain drugs, notably β blockers, nonsteroidal antiinflammatory agents, and captopril, are known to potentiate the development of hyperkalemia and render potassium replacement therapy more hazardous.

A broad consensus exists that some subgroups of patients receiving diuretics, being at special risk from hypokalemia, should be treated to replace or, if

possible, to prevent the development of any potassium deficits. Patients treated with digitalis, those in whom a massive diuresis is anticipated, and those at risk for developing hepatic coma fall under this rubric. Whether patients with known heart disease warrant this special consideration remains open to question. More questionable still is the use of potassium supplements or potassium-sparing agents to replace diuretic-induced potassium losses in patients without known heart disease and without established risk from hypokalemia. Those who argue for such "routine" potassium replacement measures are more concerned about the possible adverse cardiovascular effects of even mild potassium deficits than about the possibility of inducing life-threatening hyperkalemia (Kaplan, 1984). Those who counsel against such routine use point to our present uncertainty about the frequency of hyperkalemia as a complication of potassium replacement measures and to the paucity of evidence that mild hypokalemia is overly dangerous (Harrington *et al.*, 1982; Kassirer and Harrington, 1985).

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Chapter 17

Metabolism and Potassium

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The mechanisms by which potassium affects muscle metabolism are, in broad terms, four in number. First, potassium very likely exerts a regulatory influence on muscle blood flow during exercise. The resulting increase of muscle blood flow subserves delivery of chemical substrates for contraction and facilitates removal of heat and metabolites from the working muscle cells. A second metabolic effect relates to the role of potassium ions in carbohydrate metabolism, especially that of glycogen synthesis. A third mechanism whereby potassium affects muscle metabolism occurs indirectly by its effects on insulin secretion. The fourth general mechanism concerns the direct metabolic effect of this ion on the muscle cell itself. In this chapter, work will be described on the first two mechanisms, blood flow and carbohydrate metabolism. The remaining two considerations will be discussed.

I. THE ROLE OF POTASSIUM IONS AND THE REGULATION OF MUSCLE BLOOD FLOW

Dawes (1941) showed that infusing potassium chloride into the brachial artery induced vasodilitation and increased muscle blood flow. Realizing that physical exercise may be associated with the net release of potassium ions from skeletal muscle, Kjellmer (1961) examined potassium movement from contracting muscle cells into the interstitial fluid of isolated leg muscles of the cat during exercise. Figure 1 illustrates the essential findings of those experiments. To estimate the concentration of potassium ions in interstitial fluid, Kjellmer inserted polyethylene tubes through the upper surface of an isolated muscle. He than made slits in the bottom of the muscle so that interstitial fluid could drip by

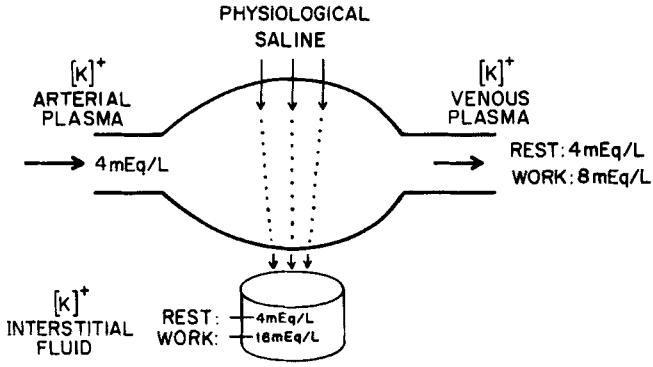

FIG. 1. Diagrammatic representation of Kjellmer's experiment showing a method to estimate interstitial fluid K concentration in skeletal muscle during exercise. The muscle was perfused with concentration in fluid collected by gravity (interstitial fluid) and venores physiological saline. While at rest, K plasma was the same as arterial plasma, indicating no net K release. During exercise, venous plasma [K] rose to 8 mEq/liter and interstitial fluid [K] rose to 16 mEq/liter, indicating net K release. Since infusing K salts at concentrated up to 16 mEq/liter dilates muscle arterioles and increases muscle blood flow, it is assumed that interstitial fluid hyperkalemia produced by exercise may play a role in exercise hyperemia.

gravity into a petri dish. The muscle was perfused with isotonic saline. The concentration of potassium in arterial plasma entering the muscle was 4 mEq/liter. While the muscle was resting, the concentration of potassium in the interstitial fluid and venous plasma effluent was also 4 mEq/liter. During electrically stimulated muscle contractions, potassium concentration in interstitial fluid rose to values ranging between 12 and 16 mEq/liter, while that in the effluent venous plasma rose to 8 mEq/liter. The observation that venous potassium concentration was considerably less than the interstitial fluid potassium concentration probably represents an effect of mixing between interstitial fluid and capillary fluid and reaccumulation of potassium ions from interstitial fluid back into muscle cells.

These observations suggested that potassium release from contracting muscle cells is probably not simply a biological accident, but rather represents a physiological mechanism contributing toward regulation of muscle blood flow during exercise.

That potassium is released from muscle cells during depolarization and formation of action potentials during exercise is well known (Hodgkin and Huxley, 1952; Hodgkin *et al.*, 1949). Although potassium ions released during muscle contraction must be transferred back to the interior of the muscle cell, under certain conditions, sufficient quantities diffuse into the capillary circulation and from there may escape into the systemic circulation so as to produce potentially dangerous hyperkalemia.

At first glance, net accumulation of potassium in venous blood sufficient to attain potentially toxic levels might be surprising in view of the exceptionally small quantity actually released. Thus, it has been estimated that only 1/100,000 of the cell's potassium ions need diffuse across the cell membrane to account for the electrical events during a single action potential (DeVoe and Maloney, 1980). Hodgkin and his associates (Hodgkin and Huxley, 1952; Hodgkin *et al.*, 1949) calculated that during a single action potential in the squid axon (which in electrochemical behavior closely resembles a striated muscle fiber), about 3×10^{-12} mol cm^2 of Na^+ and K^+ cross the cell membrane down their respective electrochemical gradients. This is equivalent to about 20,000 ions/μm^2 cell surface. However, it should be kept in mind that the foregoing estimate represents an ion flux for a single twitch. Continuous activity would result in release of corresponding larger quantities. In practical terms, two important considerations help explain why venous hyperkalemia potentially becomes a problem during exercise. First, the overall mass of muscle from which potassium ions can diffuse is very large, amounting to an average of 40% of body weight. Invaginations of the myofibrillar surface by the transverse tubular system increases the effective diffusional area enormously. Second, the conditions under which clinically important exercise hyperkalemia may occur are exactly those that, at least under experimental conditions, tend to increase outward potassium conductance of the muscle cell plasma membrane. These include metabolic exhaustion, accumulation of hydrogen ions inside the cell, and hypoxia (Gay and Stanfield, 1977). Thus, the intensity of exercise hyperkalemia correlates directly with the intensity of lactic acid accumulation and the drop of venous pH (Tibes *et al.*, 1974). Third, exhaustion of energy substrates and hypoxia can also impair regeneration of ATP so that active transport of Na and K contribute less toward reestablishing normal ionic composition of the cell. By such mechanisms, and perhaps others unknown at this time, interstitial potassium concentration may rise up to 15 mEq/liter or more during pronounced sustained contractions in both animals (Kjellmer, 1961) and man (Vyskocil *et al.*, 1983). Although venous potassium concentration during intense exercise usually rises no more than 1–2 mEq/liter (McKechnie *et al.*, 1967), under certain circumstances, venous K concentration has been observed to approach 10 mEq/liter following severe competitive exercise (McKechnie *et al.*, 1967). Such levels of hyperkalemia impose potential cardiotoxicity in athletes during exhaustive exercise or patients during massive, sustained convulsive seizures.

In experimental potassium deficiency, losses occur almost exclusively from skeletal muscle. At least in early stages, the potassium content of other more vital organs or tissues remains essentially normal (Knochel, 1978; Irvine and Dow, 1968). This apparent reservoir function of skeletal muscle, namely, the property to give up its content of potassium in order that other tissues may maintain their vital functions, might explain why skeletal muscle symptoms and

findings so commonly dominate the clinical manifestations of potassium deficiency.

Weakness, myalgias, cramping with exercise, and akathisia are common symptoms in potassium-deficient patients. Frank rhabdomyolysis and myoglobinuria have occurred when severe potassium deficiency has been produced by diuretics or when individuals moderately or severely depleted of potassium subject themselves to marked, exhaustive exercise (Knochel, 1982; Knochel *et al.*, 1972, 1978). When potassium deficiency is severe, and especially when serum potassium concentration falls below 1.5 mEq/liter, frank muscular paralysis is commonly observed. As in any electrolyte disturbance, the rapidity with which potassium deficiency and hypokalemia appear is usually directly related to the severity of symptoms. When potassium deficiency develops slowly, symptoms are generally less prominent. In such cases, there often occurs loss of other major intracellular components, including protein, so that during the process of cellular shrinkage, the cell apparently attempts to maintain a balanced composition of its critical components.

Kaliopenic myopathy has been observed in patients with severe potassium deficiency as a result of diuretic therapy, licorice ingestion, primary aldosteronism, carbenoxylone therapy for peptic ulcer disease, distal renal tubular acidosis, and inordinate losses in sweat during physical training in hot weather.

Studies conducted in this laboratory examined the possibility that potassium release from contracting muscle cells and corresponding muscle blood flow under conditions of potassium deficiency might be abnormally low (Knochel *et al.*, 1972). Accordingly, one mechanism for muscle cell injury in this condition could be ischemia. For this purpose, dogs were made potassium deficient by gavage feeding them a synthetic diet selectively deficient in potassium. Each dog was given deoxycorticosterone (DOCA), 20 mg intramuscularly once daily for the first 5 days of the depletion phase.[1] Control dogs were given the same synthetic diet containing 1 mEq K/kg body weight.

Table I shows the values for serum potassium concentration and muscle potassium content in normal and depleted dogs. Muscle potassium content declined ~50%, thus indicating severe potassium deficiency. Figure 2 shows that both potassium release and muscle blood flow increased briskly following initiation of electrical stimulation of the gracilis muscle. In contrast, potassium release showed virtually no change in the potassium-deficient animals. In addition, muscle blood flow did not rise significantly despite brisk contractions of the potassium-deficient muscle.

Additional studies were conducted on potassium-deficient dogs during exercise to determine the effects of infusing 0.15 *M* KCl into the gracilis artery at a

[1] Niedermeier and Carmichael (1962) have shown that DOCA of itself has no harmful effect on skeletal muscle in the absence of potassium deficiency.

TABLE I
POTASSIUM CONCENTRATION IN SERUM AND POTASSIUM CONTENT IN SKELETAL MUSCLE FROM NORMAL AND DEPLETED DOGS[a]

	Serum (mEq/liter)	Muscle (mEq/100 g FFDS)
Normal	4.1 (3.3–4.9)	42.7 (37.6–48.2)
K-depleted	2.3 (1.8–3.3)	22.2 (20.2–23.8)

[a] Numbers in parentheses indicate range of values found.

rate sufficient to elevate venous potassium concentration flowing from the gracilis muscle and determine its effect on muscle blood flow. Muscle blood flow increased in response to this maneuver despite the existence of potassium deficiency (Fig. 3). This supported our notion that it was decreased potassium concentration in muscle interstitial fluid that suppressed exercise hyperemia of contracting muscle in the potassium-deficient dogs.

Histologic examination of muscle samples from both normal and potassium-deficient dogs before stimulation showed no abnormality by conventional light microscopy. Samples collected immediately after stimulation showed only slight

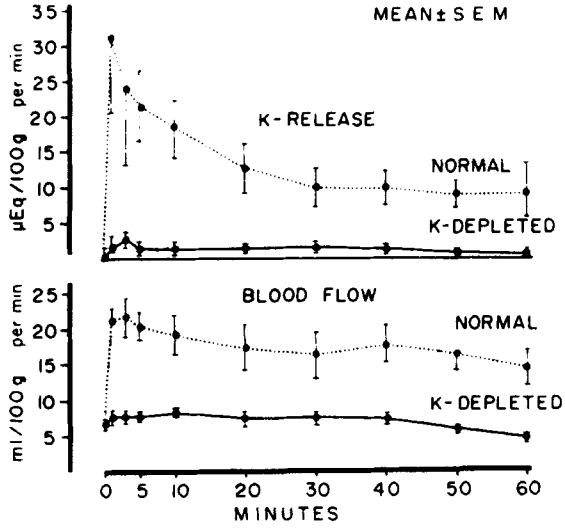

FIG. 2. Effect of stimulated exercise in the isolated gracilis muscle on K release and muscle blood flow in normal (●- - -●) and K-depleted (●——●) dogs.

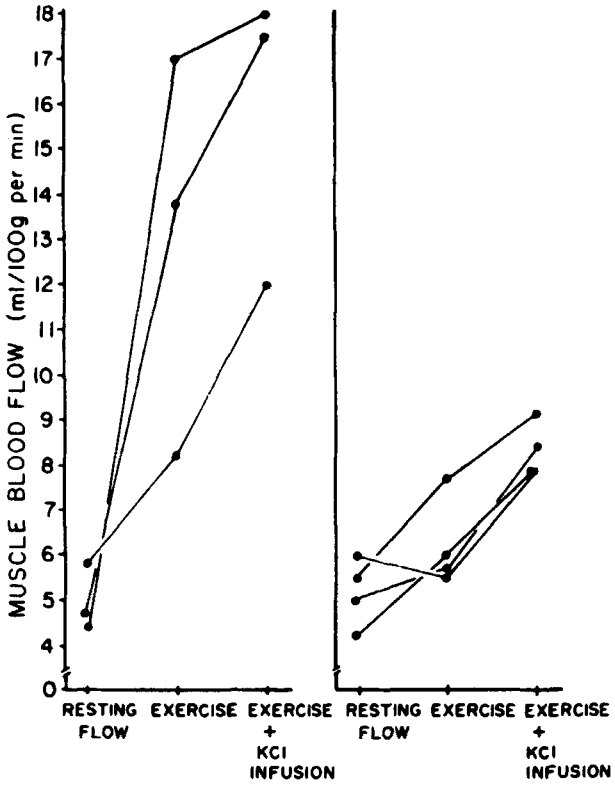

FIG. 3. Effect of KCl infusion on muscle blood flow during exercise in normal (left) and K-depleted (right) dogs.

edema. In contrast, samples collected 48 hr following completion of exercise in the potassium-deficient dogs showed frank muscle fiber necrosis with edema, hemorrhage, and disruption of structural characteristics. Samples collected at this time from normal dogs showed no abnormality.

The foregoing experimental studies suggest that potassium release and muscle blood flow are markedly impaired by potassium deficiency. This occurred despite apparently normal muscular contractions. That blood flow remains low in the presence of brisk contractions implies that there could be a much greater accumulation of metabolites and reduction of pH in the potassium-deficient muscle and perhaps even a greater degree of heat buildup than would be expected to occur in normal muscle with a free blood flow. These latter possibilities have never been examined in potassium-deficient muscle.

II. CARBOHYDRATE METABOLISM IN EXPERIMENTAL POTASSIUM DEFICIENCY

Impairment of glucose utilization and decreased insulin levels in response to hyperglycemia are hallmarks of frank potassium deficiency (Conn, 1965). In the absence of subclinical diabetes mellitus, abnormal glucose utilization in patients receiving diuretics can be either prevented or cured by prevention or reversal of potassium deficiency (Rowe *et al.*, 1980; Helderman *et al.*, 1983). Indeed, it has been shown that glucose utilization and insulin release may become normal in markedly obese patients by administration of potassium (Anderson *et al.*, 1969). In normal persons who become potassium deficient, insulin secretion is reduced, not absent; thus, fasting hyperglycemia and ketoacidosis almost never occur. If, on the other hand, frank diabetes mellitus does occur under such conditions, the patient probably had marginal islet cell function before the deficiency occurred.

Since skeletal muscle occupies such a large proportion of total body mass in health, several investigators examined the role of potassium on carbohydrate metabolism in that tissue. In a series of *in vitro* studies, Hastings and his associates clearly established the requirement of potassium for glycogen synthesis not only in muscle, but in a number of other tissues as well (Fenn, 1931; Buchanan *et al.*, 1949). They showed that if potassium ions were totally removed from an incubating solution, glycogen could not be synthesized. Gardner (1950) examined the effects of dietary potassium deprivation in adult rats and found that in very *early* potassium deficiency, there was an increase in glycogen content of both muscle and liver. In contrast, later when potassium deficiency became severe, muscle glycogen disappeared completely. Niedermeier and Carmichael (1962) studied rats with potassium deficiency induced by DOCA. They showed that muscle glycogen content disappeared. But despite DOCA, glycogen remained within normal limits if adequate potassium were provided in the diet to prevent potassium deficiency. Thus, DOCA per se does not affect glycogen synthesis.

Unfortunately, none of the studies has shown impairment of glycogen synthesis *in vitro* at potassium concentrations that would prevail in mammalian muscle cytoplasm, even in cases of severe potassium deficiency. Thus, Torres and co-workers (1966) showed that glycogen synthesis occurred at potassium concentrations *in vitro* that were clearly less than those that would prevail in the cytoplasm of muscle under conditions of severe potassium deficiency. Accordingly, Losert and his associates (Losert, 1968) proposed that either sodium ions or hydrogen ions that accumulate in potassium-deficient muscle might better explain the adverse effects of potassium deficiency on muscle glycogen synthesis. Studying muscle from potassium-deficient rats, they showed that when potassium deficiency became sufficiently advanced to cause an increase of intra-

cellular sodium, there occurred 50% inhibition of a major enzyme participating in glycogen metabolism, phosphorylase phosphatase. This enzyme inactivates the active phosphorylase (phosphorylase a) by dephosphorylation. They also conducted *in vitro* studies and confirmed that sodium at a concentration of 25 mEq/liter, which is the concentration usually found in potassium-deficient cells, inhibited the enzyme. Biologically, the net result of this inhibition would be an enhanced rate of glycogenolysis.

A criticism of published works concerning potassium deficiency and glycogen synthesis concerns the experimental preparation of the animal. Characteristically, animals fed potassium-deficient diets become anoretic. In rats fed potassium-deficient diets, food consumption declines and growth rates become markedly depressed (Gardner, 1950). In adult dogs, weight loss occurs soon after beginning potassium-deficient diets. Such events imply that food deprivation of itself could be responsible for the eventual decline in tissue content of glycogen. To assess the interaction of potassium and glycogen synthesis, it thus becomes necessary to feed the animal by gavage in order to circumvent the possible effects of malnutrition. The following study examined such possibilities.

A. Methods

The effect of potassium deficiency on muscle glycogen content was studied in healthy mongrel dogs. Six control animals were fed a potassium-deficient diet (Nutritional Biochemicals, Inc.) to which 1 mEq/kg of potassium was added daily. Additional groups, each containing 6–8 animals, were fed the deficient diet for the duration of the study. The diet was administered by gavage in a quantity of 30 g/kg body weight each day. The experimental groups of animals also received DOCA in a dosage of 20 mg/day for the first 5 days of the depletion phase. Sodium chloride, 10 mmol/kg/day, was added to the diet in both groups of animals. Approximate potassium balance was estimated from recovery of potassium in daily urine collections and assumed losses of 1 mEq daily in feces (Lemieux *et al.*, 1964). Studies on the depleted dogs were conducted when estimated body potassium was reduced by ~25–32%.

During each experimental procedure, all dogs were anesthetized with sodium pentobarbital, 30 mg/kg, and intubated with an endotracheal tube. Large bore polyethelene catheters were placed in the right juglar vein and the left common carotid artery for purposes of sampling, monitoring of systemic blood pressure, administration of drugs, and collections of venous blood from the gracilis muscle. Sodium heparin was used throughout each experiment for anticoagulation.

In order to quantitate uptake and metabolism of glucose by skeletal muscle at rest and during exercise, the gracilis preparation as described by Skinner and Powell (Skinner and Powell, 1967) was utilized except that the main artery to the

gracilis muscle was not disturbed. The nerve to the gracilis muscle was then isolated and cut. After denervation and stabilization of blood flow for a minimum of 20 min, faradic stimulation of the distal stump of the gracilis nerve was begun at a rate of 5/sec using supramaximal voltage pulses of 0.175-sec duration. Arterial and venous blood samples were collected across the gracilis muscle at rest and during work. They were immediately placed into cold perchloric acid for measurement of glucose and lactate. Both of the latter substances were measured by standard enzymatic procedures.

Blood flow rates were determined with a Gilford Photoelectric Drop Counter. During the experiment, measurements were obtained at 1-min intervals.

Additional studies were conducted on healthy mongrel dogs to determine the effect of potassium deficiency on the glycogen supercompensation phenomenon (Bergstrom and Hultman, 1966). The latter term describes the rebound accumulation of glycogen in skeletal muscle up to levels fivefold higher than those measured before a single episode of exhaustive, glycogen-depleting exercise. Muscle samples were collected by needle biopsy from the lateral thigh. Local cutaneous anesthesia was induced over the biopsy site using xylocaine. The dogs were given innovar before each procedure for general sedation. The animals showed no evidence of pain while 5 to 10-mg samples of muscle were removed using a small, hollow needle biopsy instrument. Assuming that the glycogen content of the lateral muscles of the thigh are equal on left and right sides, a control sample was collected for measurement of glycogen content from the resting left leg. The right lateral thigh was then shaved and a superficial electrode attached to the skin overlying the right lateral thigh muscles. Contractions were induced electrically at a rate of 5/sec to the point of exhaustion. Muscle samples for glycogen content were again collected on a second, third, and fifth day following exhaustive exercise. Studies were conducted on dogs before and after potassium depletion. Glycogen in muscle was measured by the method of Lo *et al.* (1970).

Studies were also conducted to quantitate capacity for work. For this purpose, the gracilis preparation described in the preceding paragraph was utilized, except for one difference. Instead of allowing the distal tendon on the gracilis muscle to remain attached to the bone, the tendon was disconnected and firmly tied to a cable holding a 1.5-kg weight. The cable was directed over the wheel of a potentiometer. The potentiometer was connected to a graph recorder. The quantity of work was estimated as the area under the curve inscribed for each minute to the point of exhaustion. The work capacity index was arbitrarily defined as that point at which the recorded work fell to 50% of the initial inflexion. Before electrical stimulations were begun on the gracilis nerve, the cable attached to the gracilis tendon was adjusted to the point at which the muscle was stretched exactly to its full length of relaxation. Electrical stimuli were applied at the rate of 5/sec.

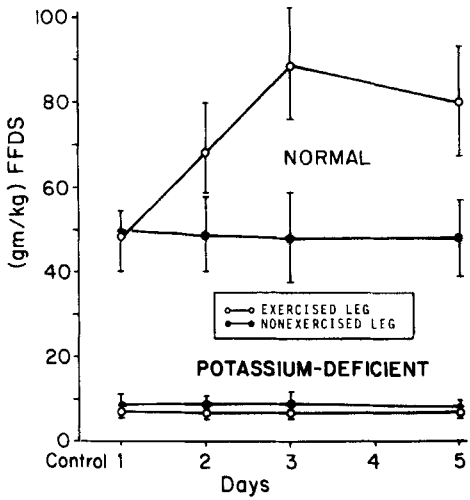

FIG. 4. Upper: Muscle glycogen content rises (supercompensation) after a single bout of exhaustive exercise in the normal dog in the exercised, but not the unexercised muscle. In potassium-deficient dogs (lower), baseline values for muscle glycogen become very low and show no change after exercise.

B. Results

Figure 4 illustrates comparative values for muscle glycogen content in normal and potassium-deficient dogs. In addition, the figure shows sequential changes in muscle glycogen content following a single bout of exhaustive exercise. In normal dogs, the average muscle glycogen content averaged 49.2 ± 4.1 g/kg fat free dry solids (FFDS). In potassium-deficient dogs, this fell to an average value of 7.7 ± 1.2 g/kg FFDS. Sequential measurements of muscle glycogen content following a single bout of exhaustive exercise in normal dogs showed a value of 69.3 ± 9.6 g/kg of FFDS on the first day, 89.3 ± 11.5 g/kg FFDS on the third day, and an average value of 80.9 ± 8.0 g/kg FFDS on the fifth day. Muscle glycogen content in the unexercised leg showed essentially no change. In contrast to these observations, serial measurements of muscle glycogen content at the same intervals in potassium-deficient dogs showed no evidence for supercompensation. Table II shows values for glucose uptake and lactate output across the gracilis muscle in normal and potassium-deficient dogs. Resting glucose uptake in normal dogs was 88 ± 7 μmol/kg/min. In potassium-deficient dogs, the value was 91 ± 9 μmol/kg/min. These values are not significantly different. Under conditions of stimulated work, glucose uptake in normal dogs was 118 ± 14 μmol/kg/min. In potassium-deficient dogs, glucose uptake during stimulated work was 116 ± 9 μmol/kg/min. Resting lactate output from the normal gracilis

TABLE II
GLUCOSE UPTAKE AND LACTATE OUTPUT IN NORMAL AND K-DEFICIENT DOGS AT REST AND DURING EXERCISE

	State	Normal	*P*	K deficiency
Glucose uptake (μmol/kg/min)	Resting	88 ± 7		91 ± 9
	Working	118 ± 14		116 ± 9
Lactate output (μmol/kg/min)	Resting	26 ± 5		32 ± 7
	Working	610 ± 80	<0.01	240 ± 60

muscle was 26 ± 5 μmol/kg/min in the normal dogs and 32 ± 7 μmol/kg/min in the potassium-deficient dogs. During electrically stimulated work, lactate output in normal dogs rose markedly to 610 ± 80 μmol/kg/min. In contrast, lactate output was only 240 ± 60 μmol/kg/min in the potassium-deficient animals. Analysis of these data indicate that lactate production considerably exceeded the quantity that could have been provided by glucose uptake by the muscle under normal conditions. This infers a major utilization of glycogen and its conversion to lactate under conditions of work in the normal animals. In sharp contrast, at a glucose uptake of 116 mmol/kg/min by the K-deficient muscle, one would anticipate a maximum lactate production of twice this value if no glycogen were available for utilization. Of interest, the lactate output was almost exactly predictable from glucose uptake, indicating that the available glycogen in the potassium-deficient muscle was not utilized. In addition, these data indicate that there was no apparent defect in glucose utilization by potassium-deficient muscle. They also indicate that the phenomenon of glycogen supercompensation is essentially eradicated by potassium deficiency.

Figure 5 illustrates data on work capacity of normal and potassium-deficient dogs. In terms of arbitrary work units, as assessed by examination of 6 normal animals, it can be seen that in 3 of the animals, muscle preparations essentially lost their responsiveness to electrical stimuli within a period of 4 min. In the remaining 3 animals, the decline of responsiveness was more gradual and fell by an average value of about 60% after 12 min. In sharp contrast to the observations on normal animals, work unit intensity was extremely variable in the potassium-deficient animals and appeared to show an inverse correlation with the degree of potassium deficiency. In the lower part of the right panel, it is seen that 3 animals with potassium deficits ranging from 27% to 32.5% showed only minimal work production in the early period of stimulation and a rpaid falloff within a period of 2–6 min to virtual inexcitability. The 3 remaining dogs with deficits ranging between 24.7% and 26.2% showed values for work capacity that were normal or even higher than normal. Table III shows values expressing these data in terms of maximal strength and endurance. Work capacity was expressed as gram/min-

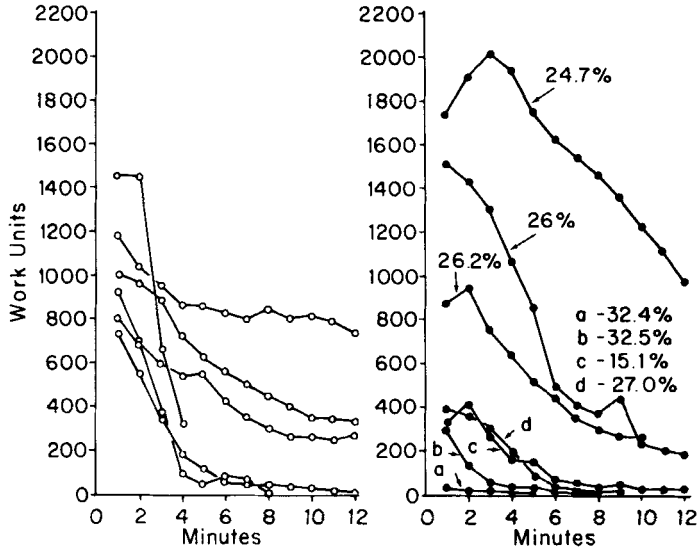

FIG. 5. A comparison of work capacity of normal (left) and potassium-deficient (right) skeletal muscle. The percentage values refer to the estimated potassium deficit that existed in each dog when the study was conducted.

ute/gram muscle as determined from the deflections on the potentiometer while lifting a weight during contractions. A maximum strength of normal muscle was 1022 g/min/g muscle compared to a value of 811 in the potassium-deficient muscle. These values were not significantly different. However, there was a significantly lesser degree of endurance in the potassium-deficient animals. Endurance expressed as half-time from the initial work intensity compared to its value at 12 min showed a value of 6.4 in the normal animals and 3.2 in the potassium-deficient animals. These two values were significantly different. When both groups were expressed as average values of percentage of strongest contractions compared to time (Fig. 6), there was no difference. One can con-

TABLE III
A COMPARISON OF WORK CAPACITY AND ENDURANCE OF GRACILIS MUSCLES FROM NORMAL AND K-DEFICIENT DOGS

	Normal	P^a	K deficiency
Maximum strength (g/min/g muscle)	1022 ± 261	NS	811 ± 749
Endurance (T/2; min)	6 ± 4	<0.05	3 ± 2

[a] NS, Not studied.

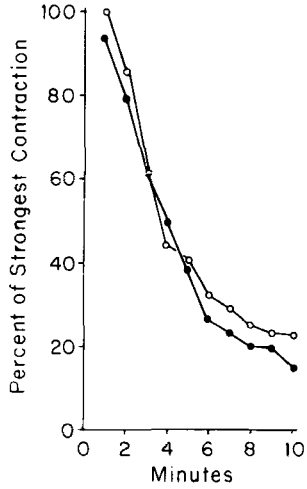

FIG. 6. A comparison of muscular contractile force in normal (○) and K-deficient (●) dogs.

clude from these studies that potassium deficiency does not necessarily impair contractile strength but probably, because of a decreased supply of glycogen, impairs endurance.

III. INSULIN METABOLISM IN POTASSIUM DEFICIENCY

Conn (1965) was the first to make the observation that insulin levels in serum were diminished in response to hyperglycemia in patients with potassium deficiency due to primary aldosteronism. In those patients, correction of the potassium deficit returned the insulin values to normal. A number of studies have been conducted since that time both in animals and man showing that potassium deficiency induced by a variety of causes reduces insulin release from the pancreas and indicates that carbohydrate intolerance in potassium deficiency appears to be the result of inadequate insulin release. Although earlier studies in patients with hypokalemia associated with diuretic therapy suggested that the diuretic itself was responsible for impaired carbohydrate utilization (Losert, 1968), this has not been borne out by subsequent studies using glucose clamp techniques. Rowe and his associates (Rowe *et al.*, 1980) studied the effects of mild potassium deficiency in 7 young men. Potassium deficiency was induced by ingestion of a potassium binding resin which resulted in a deficit ranging between 1.0% and 8.4%. Even such a slight degree of potassium deficiency resulted in a decline of glucose metabolism by 27.4%. This was associated with a decrease in plasma insulin response to sustained hyperglycemia. Of importance, potassium

depletion had no effect on tissue sensitivity to insulin. More recently, Helderman and associates (1983), using the same glucose clamp technique, showed that potassium deficiency resulting from hydrochlorothiazide therapy was associated with a diminished pancreatic β cell response to glucose. On the other hand, when potassium deficiency was prevented by supplementation with potassium chloride, insulin secretion and carbohydrate utilization remained within normal limits.

In our own studies examining muscle utilization of glucose in potassium-deficient dogs, as described in Section II, we found that glucose utilization by skeletal muscle was normal at rest as well as during exercise. Exercise normally suppresses insulin release from the pancreas (Bottger *et al.*, 1971) so that utilization of glucose by skeletal muscle during exercise is not apparently insulin dependent.

IV. KALIOGENIC HYPERMETABOLISM

In 1931, Fenn observed that exposure of muscle tissue to a high potassium concentration increased its oxygen consumption. Figure 7 illustrates this observation. Studies conducted by other investigators since that time showed that oxygen consumption. lactate production (Kaye and Mommaerts, 1960), and heat

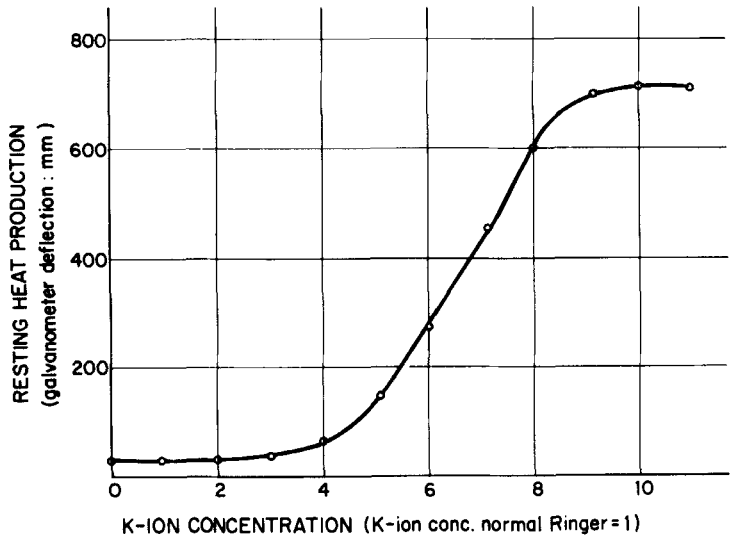

FIG. 7. The effect of ambient potassium concentration on resting heat production in skeletal muscle.

production increased when the membrane potential of the cell depolarized from −90 mV to about −70 mV (Erlij, 1982) and external [K] was raised from 4 to about 9 or 10 mEq/liter. The relationship between oxygen uptake, resting membrane potential, and potassium concentration in surrounding fluid is shown in Fig. 8. This effect occurs independently of muscle contraction. It appears at a considerably more negative potential and much lower extracellular K concentration than potassium-induced contractures (Hodgkin and Horowicz, 1960). Although at one time this was thought to be the result of reducing the resting membrane potential (Horowicz and Gerber, 1965), it is now known that the increase of respiration is dependent upon release of Ca^{2+} ions into the sarcoplasm (Kaye and Mommaerts, 1960; Novotny and Viskocil, 1966; VanderKloot, 1967). Thus, Ca^{2+} is thought to stimulate respiration. The respiratory rate of the muscle (Fig. 8) rises ~10 times above control values at [K]° of 15–20 mEq/liter. Erlij and his associates (Erlij *et al.*, 1982) estimated that calcium release into the sarcoplasm was 3.4×10^{16} ions/sec/cm^3 in response to increasing [K]° from 2.5 to 20 mEq/liter. The corresponding O_2 utilization rose from 2.7 to 19.75 μmol O_2 g^{-1} hr^{-1}. The estimated quantity of calcium ions released in response to hyperkalemia is about 250 times smaller than the calculated Ca^{2+} flux (1.2×10^{19} ions/sec/cm^3) necessary for full contractile activation of skeletal muscle. Kaliogenic hypermetabolism is also associated with activation of

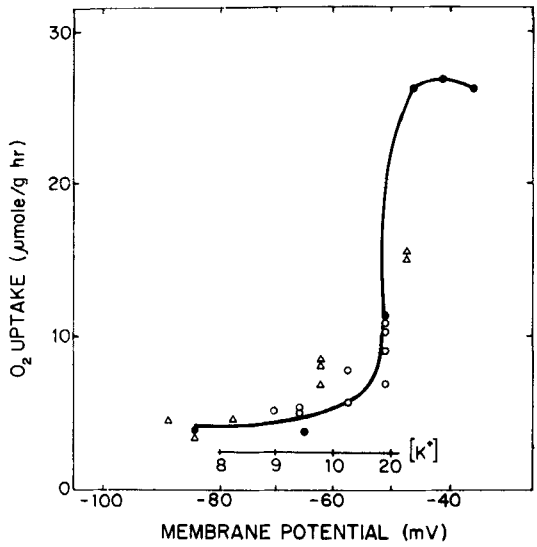

FIG. 8. The effect of changing extracellular K concentration on resting membrane potential and oxygen uptake in skeletal muscle.

muscle phosphorylase, increased activity of phosphofructokinase, and enhanced glycolysis (Kaye and Mommaerts, 1960).

So long as [K]° does not exceed 20 mEq/liter, the high respiratory rate is sustained. When [K]° exceeds 20 mEq/liter, there occurs an additional transient burst followed by a decline. The respiratory stimulation by K can be blocked by depolarizing agents such as procaine (Novothny *et al.*, 1962) or high concentrations in extracellular fluid of several divalent cations including strontium, calcium, magnesium, manganese, and cobalt (VanderKloot, 1967; Hill and Howarth, 1957). The mechanism whereby divalent cations in the extracellular fluid reduce kaliogenic hypermetabolism is unclear. Although it could result from the tendency of these ions to move the activation potential toward zero, this has not been proved. The observation that muscle respiration rises whether the cells are exposed to elevated concentrations of potassium, cesium, or rubidium indicates that the effect is not specific for K, but rather for depolarization of the cell. Hyperkalemic hypermetabolism is not dependent upon sodium (VanderKloot, 1967), since the respiratory effect occurs when sodium is replaced with choline. Furthermore, studies by Horwicz and Gerber (1965) showed that ouabain-sensitive sodium efflux is stimulated only 2–3 times above normal when [K]° is increased to 15 m*M*.

Kaliogenic hypermetabolism is markedly reduced by dantrolene and deuterium, substances that are known to impair calcium release from sarcoplasmic reticulum in response to deploralization (Erlij *et al.*, 1982). Neither substance affects membrane potential or other electrical properties of muscle (Gronert, 1980). Dantrolene completely abolished the kaliogenic hypermetabolism in response to K^+ concentrations up to 15 mEq/liter. The effect of caffeine, known to increase muscle respiration by promoting release of calcium from sarcoplasmic reticulum, is also blocked by dantrolene. Increasing concentration of Cl^- in incubating solutions or replacing chloride with sulfate potentiates the respiratory response to hyperkalemia by a factor of three (Erlij *et al.*, 1982).

Malignant hyperthermia is a rare disorder characterized by the rapid appearance of hyperkalemia, increased CO_2 production, acidosis, hypoxia, a rapid increase of body temperature to values as high as 44.4°C (112°F), rhabdomyolysis, and death. It is usually precipitated by depolarizing drugs or general anesthetics (Gronert, 1980). It is usually, but not always, associated with muscle rigidity during induction of anesthesia. Although its cause is not clearly understood, increasing evidence suggests that it represents an abnormality of the excitation–contraction mechanism in skeletal muscle in which calcium release is triggered by depolarizing agents or destabilization of sarcoplasmic reticulum calcium storage. That dantrolene prevents as well as aborts attacks of malignant hyperthermia strongly supports the notion that it is mediated by a disturbance in the excitation–contraction mechanism. That it may indirectly be related to hyperkalemic respiratory stimulation seems quite possible.

Since biological events are seldom without purpose, one might speculate that

K release from muscle cells during exercise might have an additional assignment rather than simply that of a vasodilator. If, as discussed previously, interstitial K concentration in exercising muscle rises to 10–15 mEq/liter, perhaps interstitial hyperkalemia could play a role in the poorly understood phenomenon of "warm-up" and its salutory effect on exercise performance in normal persons or the "second wind" phenomenon in patients with myophosphorylase deficiency. Relevant to this notion is the observation by VanderKloot that K-stimulated respiration activates muscle phosphorylase and increases activity of phospho-fructokinase (VanderKloot, 1967).

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Part IV

Potassium Transport in Muscle and Colon

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Chapter 18

Effects of Potassium Deficiency on Na,K Homeostasis and Na^+,K^+-ATPase in Muscle

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I. EFFECTS OF K DEFICIENCY ON Na,K DISTRIBUTION *in Vivo*

K deficiency induced by the administration of K-deficient diet to rats leads to a rapid decrease in plasma K. This is associated with a progressive loss of K from the skeletal muscles and to a minor extent from the heart. In contrast, the brain, liver, and erythrocytes maintain almost normal K contents for up to 8 weeks followed by a terminal decrease (see Fig. 1 and Heppel, 1939; Bradbury and Kleeman, 1967; Chan and Sanslone, 1969; Akaike, 1979; Nørgaard *et al.*, 1981; Kjeldsen *et al.*, 1984a). The selective loss of K from muscle cells is associated with an almost equivalent increase in Na content, and in rat skeletal muscle, the intracellular Na concentration was found to undergo an up to 8-fold rise (Kjeldsen *et al.*, 1984a). In keeping with the larger requirement for K in young as compared to mature animals (Miller, 1926), young rats lost around 40% of total K after 1 week of K depletion, whereas the decrease was only around 20% in mature rats. These changes are rapidly reversible following the readministration of KCl via stomach tube, and within 2 hr muscle Na,K contents return to the control levels (Kjeldsen *et al.*, 1985a).

K deficiency induced by diuretics and mineralocorticoids gave a similar pattern of changes in plasma and tissue Na,K contents, but the anomalies were less pronounced (see Fig. 4). The effects of diuretics on muscle Na,K contents were offset by K supplement (Kjeldsen *et al.*, 1984a).

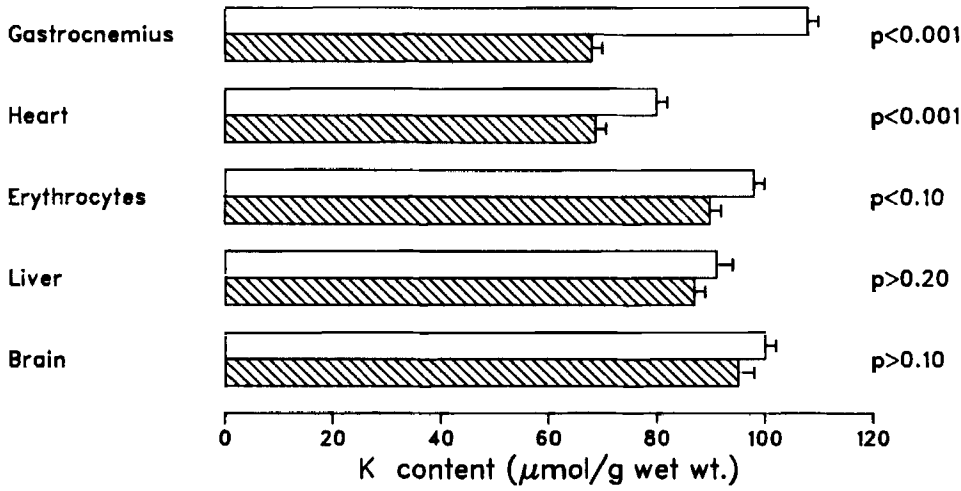

FIG. 1. Effects of K deficiency on tissue K contents. Rats that were 12 weeks old, were maintained for a further 8 weeks on K-deficient fodder containing 0.75 mmol K per kilogram (0.3% of the K content of the standard fodder). The animals were killed by decapitation and tissue samples taken for K determination by flame photometry (for details, see Kjeldsen *et al.*, 1984a). Columns indicate the mean of observations on 3–8 animals, with bars denoting SE. (□), Control; (▨), K-depleted rats.

II. EFFECTS OF K DEFICIENCY ON [^{3}H]OUABAIN BINDING AND Na^+, K^+-ATPase IN ISOLATED CELLS

It is well known that the exposure of cells to a low extracellular K concentration *in vitro* leads to a progressive loss of intracellular K associated with a gain of Na. The maintenance of constant intracellular Na,K contents depends on, among other things, the activity and the concentration of Na^+,K^+-ATPase in the plasma membrane. An increase in the intracellular Na/K concentration ratio will lead to an activation of the Na,K pump, possibly allowing the establishment and maintenance of a new steady state. Studies with several types of cultured cells have shown that following this early adaptation to low extracellular K, there is a long-term adaptation consisting of the synthesis of more Na,K pumps, favoring the reestablishment of the initial levels of Na,K contents. Thus, in HeLa cells (Boardman *et al.*, 1972; Pollack *et al.*, 1981), fibroblasts and cardiac myocytes (Werdan *et al.*, 1984), and avian myocytes (Fambrough and Bayne, 1983), the exposure to culture medium containing 1–3 m*M* K led to a progressive increase in [^{3}H]ouabain binding capacity, Na^+,K^+-ATPase activity, or ouabain-suppressible K uptake.

This increase in the population of Na,K pumps has been considered as a

general counterregulatory response to the elevated intracellular Na and has indeed also been exemplified in several studies on intact tissues and organisms. Thus, in erythrocytes obtained from K-deficient human subjects or animals, the activity of Na^+,K^+-ATPase and the [^{3}H]ouabain binding capacity is increased (Chan and Sanslone, 1969; Erdmann *et al.*, 1984).

III. EFFECTS OF K DEFICIENCY ON [^{3}H]OUABAIN BINDING IN SKELETAL MUSCLE *in Vivo*

In contrast to the up-regulation described above, the muscle cells of the intact organism respond to K deficiency by a decrease in [^{3}H]ouabain binding capacity (Nørgaard *et al.*, 1981). In the soleus of mature rats, this decrease could be detected within 3 days after the start on K-deficient fodder and continued for several weeks until around 60% of the [^{3}H]ouabain binding sites had been lost (Fig. 2). In keeping with the earlier onset of K deficiency and its associated symptoms, the most pronounced effect was seen in young animals. Thus, in

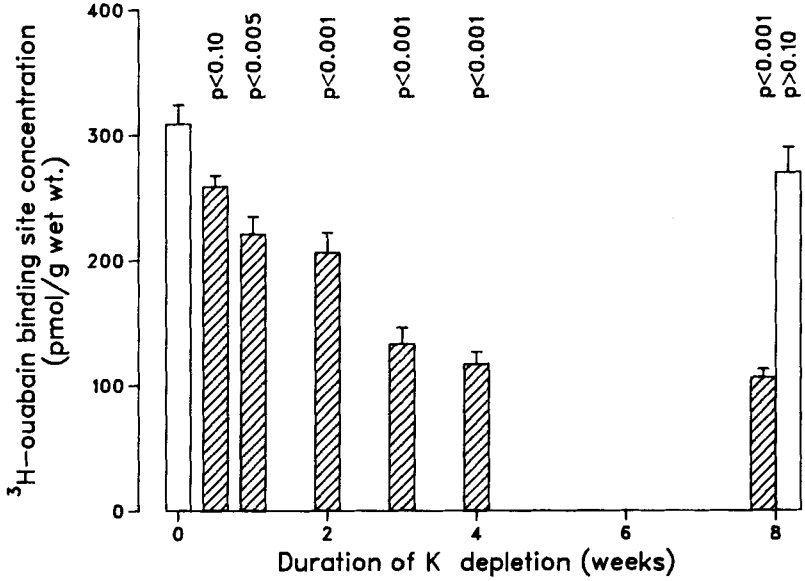

FIG. 2. Time course of changes in [^{3}H]ouabain binding capacity in rat soleus muscles during K deficiency. Rats that were 12 weeks old were maintained on K-deficient fodder for a further 3 days to 8 weeks. At the indicated intervals, the animals were killed and the lateral strips of the soleus muscle prepared for determination of the concentration of [^{3}H]ouabain binding sites. Columns indicate the mean of observations on 4–12 animals, with bars denoting SE (for details, see Kjeldsen *et al.*, 1984a). (□), Control; (▨), K-depleted rats.

young rats a decrease of 78% was seen after 4 weeks, whereas the corresponding decrease in mature rats was 34% (Kjeldsen *et al.*, 1984a). Measurements performed on muscles containing either mainly red or white fibers indicate that the phenomenon is general and not restricted to certain fiber types. Thus, in 4-week-old rats, K depletion for a further 1 week induced a decrease of 32–51% in soleus and 33–48% in extensor digitorum longus (edl) (Nørgaard *et al.*, 1981; Clausen *et al.*, 1983; Kjeldsen *et al.*, 1984a). Studies with mice and guinea pigs gave similar results (Kjeldsen *et al.*, 1984a; Brown *et al.*, 1986), indicating that the response is not species specific. As the [^{3}H]ouabain binding site concentration in skeletal muscles of rats, mice, and guinea pigs shows major variations with age (Kjeldsen *et al.*, 1982; Kjeldsen *et al.*, 1984b), a precise matching with respect to age between K-depleted and control groups is essential, especially in young animals.

Studies of the time course of [^{3}H]ouabain binding to rat soleus muscles showed that the decrease induced by K deficiency could not be ascribed to a reduction in the rate of binding (Kjeldsen *et al.*, 1984a). Kinetic analysis showed that K deficiency was not associated with any decrease in the affinity of the [^{3}H]ouabain binding sites. As in the controls, only one population of binding sites could be discerned (Fig. 3).

In muscle, the concentration of [^{3}H]ouabain binding sites can either be measured using intact fibers, where energy metabolism is maintained (Clausen and Hansen, 1974), or in biopsies (Nørgaard *et al.*, 1983) or necropsies (Nørgaard *et al.*, 1985a) incubated in a vanadate-containing medium so as to facilitate ATP-independent binding. Direct comparison of [^{3}H]ouabain binding to intact muscle fibers and biopsies taken from the contralateral muscle of the same animals showed the same effects of K deficiency (Nørgaard *et al.*, 1983). Therefore the decreased [^{3}H]ouabain binding capacity is not likely to be the result of impaired cellular ATP production or structural integrity.

The effect of K deficiency on [^{3}H]ouabain binding capacity in skeletal muscle was not only seen in animals on low K intake, but also when the net loss of K was augmented by the administration of diuretics or a mineralocorticoid (Kjeldsen *et al.*, 1984a).

Following the readministration of K to K-deficient rats, full recovery of the [^{3}H]ouabain binding capacity was not achieved before 6 days, and the time course of reappearance of [^{3}H]ouabain binding sites indicates that this is the result of resynthesis of receptors (Kjeldsen *et al.*, 1984a). Since plasma and tissue K contents were normalized within a few hours after the readministration of K, it can be assumed that the reduction in [^{3}H]ouabain binding capacity is not the direct outcome of hypokalemia. This conclusion is further supported by the observation that K depletion performed by incubating isolated soleus muscles for 3 hr in K-free buffer caused no significant change in [^{3}H]ouabain binding capacity (Kjeldsen *et al.*, 1984a).

It should be noted that in young rats, loss of muscle K and a decrease in

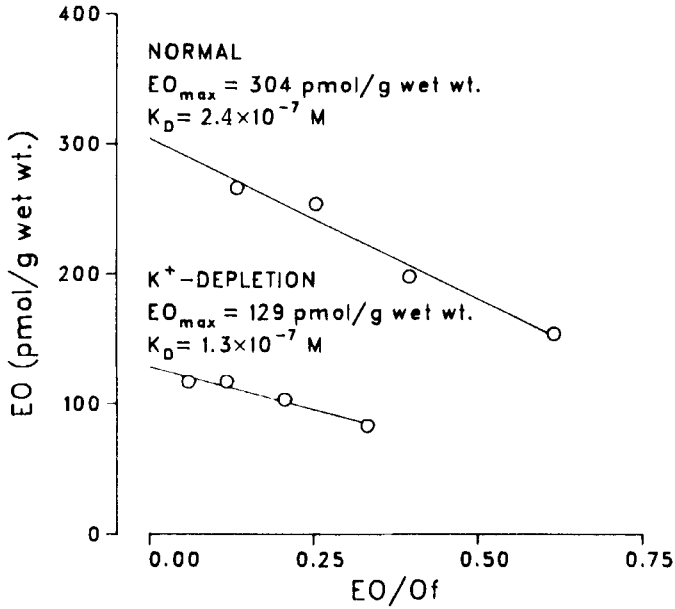

FIG. 3. The effect of K deficiency and the concentration of ouabain on the binding of [^{3}H]ouabain in rat soleus muscles. Rats that were 12 weeks old were maintained on K-deficient fodder for a further 3 weeks. The [^{3}H]ouabain binding was measured *in vitro*, incubating intact muscle fibers at 2.5×10^{-7}–2×10^{-6} mol/liter ouabain. The "bound" (EO) versus "bound/free" (EO/Of) [^{3}H]ouabain is plotted for K-deficient and age-matched control rats. Each point represents the mean of observations on 4 animals. The lines were constructed using linear regression analysis, and the calculated values for the intercepts with the ordinate (EO_{max}) as well as the apparent dissociation constants (K_D) are indicated.

[^{3}H]ouabain binding sites were seen when the K content of the fodder was reduced to less than 69 mmol/kg. Mature rats tolerated a somewhat larger reduction without developing any measurable changes or symptoms. On the other hand, loading with excess K in the fodder (up to 1350 mmol/kg) or via a stomach tube (36 mmol K/kg body weight/day) gave no significant change in muscle Na,K contents or the concentration of [^{3}H]ouabain binding sites (Kjeldsen *et al.*, 1984a).

IV. EFFECTS OF K DEFICIENCY ON Na$^+$,K$^+$-ATPase ACTIVITY IN SKELETAL MUSCLE CELLS

The detection of quantitative changes in the total Na$^+$,K$^+$-ATPase activity of muscle cells is difficult for three major reasons: (1) The presence of several other ATPases gives rise to a considerable background activity; (2) attempts to purify

the Na^+,K^+-ATPase usually only allow the recovery of a few percent of the initial activity, leaving considerable uncertainty about the total quantity of enzyme (Jones and Besch, 1984; Nørgaard *et al.*, 1985b); and (3) the formation of inside-out vesicles or other structural changes during the preparation procedures may imply that the final purified material used for quantitative evaluation is not representative. Measurements of K-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity, which is closely associated with and correlated to the Na^+,K^+-ATPase activity, indicate that these problems can be circumvented. Thus, the total 3-*O*-MFPase activity in crude homogenates of rat hind limb muscles was found to be closely correlated to [^{3}H]ouabain binding capacity (Nørgaard *et al.*, 1984). Furthermore, in gastrocnemius muscle from young rats, K depletion for 1 week led to a reduction in 3-*O*-MFPase activity of 37% (Kjeldsen *et al.*, 1984a). Hence, the measurements of 3-*O*-MFPase confirm the effect of K depletion on [^{3}H]ouabain binding capacity and indicate that the decrease reflects a reduction in the concentration of Na^+,K^+-ATPase.

V. POSSIBLE MECHANISMS FOR THE DOWN-REGULATION OF [^{3}H]OUABAIN BINDING SITES WITH K DEFICIENCY

Following the administration of K-deficient fodder, the relative decrease in plasma K is considerably larger than that in muscle K. Obviously, the loss of K from the extracellular phase precedes and causes the loss of intracellular K. The possible cause–effect relationship between loss of muscle K and down-regulation of [^{3}H]ouabain binding sites is less clear. In K-deficient animals, the relative decrease in skeletal muscle K content is roughly the same in different fiber types. Therefore, the gastrocnemius muscle, being composed of a mixture of fiber types, was used to quantify the changes in muscle K content during K deficiency. Measurements on soleus and edl muscles obtained from mice, guinea pigs, and rats of different ages showed that K deficiency reduced the concentration of [^{3}H]ouabain binding sites. As shown in Fig. 4, this is in all instances associated with a drop in the K content of the gastrocnemius muscles prepared from the same animals. The data on soleus muscles from 12-week-old rats maintained on the K-depleting regime for from 3 days to 8 weeks showed a linear correlation ($r = 0.99$, $P < 0.001$). For the same group of animals, K deficiency induced an even larger relative change in intracellular Na concentration and, as shown in Fig. 5, this is also related to the decrease in [^{3}H]ouabain binding site concentration. It is possible that the reduction in [^{3}H]ouabain binding site concentration is secondary to the increased intracellular Na/K concentration ratio. The problem is that the increased intracellular Na/K concentration ratio might as well be the outcome of a reduction in the population of Na,K pumps. This possibility,

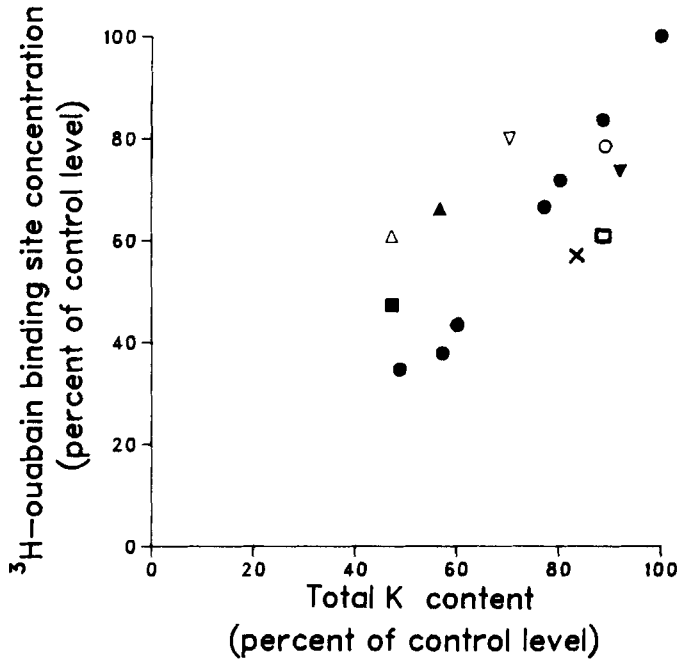

FIG. 4. Relationship between the changes in the total K content of gastrocnemius muscle and the concentration of [^{3}H]ouabain binding sites in edl (indicated) or soleus muscle of the same animals. The concentration of [^{3}H]ouabain binding sites was measured *in vitro* on intact muscle fibers. The K content was determined using flame photometry (for details, see Kjeldsen *et al.*, 1984a). The symbols indicate the following experimental conditions: (●), 12-week-old rats given K-deficient fodder for a further 3 days to 8 weeks; (■), 4-week-old rats given K-deficient fodder for a further 1 week; (□), 4-week-old rats given fodder containing 69 mmol K/kg and fluorohydrocortisone (300 μg/kg/day) for a further 1 week; (▼), 4-week-old rats given fodder containing 69 mmol K/kg and bendroflumethiazide (40 mg/kg/day) for a further 1 week; (×), 4-week-old rats given fodder containing 69 mmol K/kg and bumetanide (250 mg/kg/day) for a further 1 week. In order to obtain a K-depleting effect of diuretics or adrenal steroid, the K content of the fodder was reduced from 262 to 69 mmol/kg. This reduction per se caused no significant change in the Na,K contents or [^{3}H]ouabain binding site concentration in muscle; (○), 4-week-old rats denervated at the sciatic level and kept on normal fodder for a further 1 week; (△), 4-week-old rats given K-deficient fodder for a further 1 week (edl); (▲), 7-week-old mice given K-deficient fodder for 1 week (edl). (▽), 3-week-old guinea pigs given K-deficient fodder for 1½ weeks. Each point represents the mean of observations on 4–12 animals of K content as well as [^{3}H]ouabain binding site concentration.

however, seems less likely in view of the fact that following the reestablishment of normal plasma K, intracellular Na,K contents could be normalized within a few hours even though the concentration of [^{3}H]ouabain binding sites remained low (Kjeldsen *et al.*, 1984a).

It seems likely, therefore, that at least during the initial phase of K deficiency,

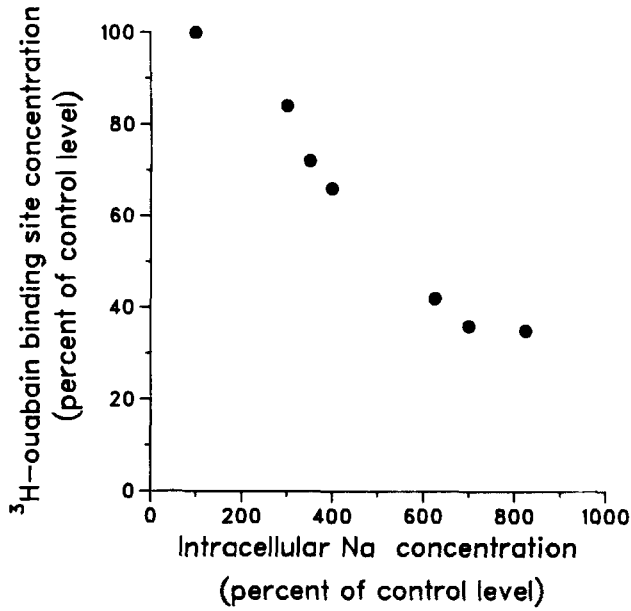

FIG. 5. Relationship between changes in intracellular Na concentration in gastrocnemius muscle and concentration of [^{3}H]ouabain binding sites in soleus muscles from 12-week-old rats given K-deficient fodder for a further 3 days to 8 weeks. The concentration of [^{3}H]ouabain binding sites was measured *in vitro* using intact muscle fibers. The Na content was determined using flame photometry. For details, see Kjeldsen *et al.*, 1984a. Each point represents the mean of observations on 4–12 animals of intracellular Na concentration as well as [^{3}H]ouabain binding site concentration.

the increase in intracellular Na/K concentration ratio can directly or indirectly elicit a reduction in the concentration of [^{3}H]ouabain binding sites in muscle cells. This adaptation could either be mediated by the nervous supply or a circulating endocrine factor. The first possibility was ruled out by the observation that following denervation, both soleus and edl muscles responded to K deficiency with the same reduction in [^{3}H]ouabain binding site concentration as the contralateral innervated muscles and that denervation does not prevent the recovery in [^{3}H]ouabain binding capacity during K repletion (Clausen *et al.*, 1983). The possible contribution of endocrine factors is more difficult to exclude, but might be further tested by culturing isolated myocytes in plasma obtained from K-depleted animals.

Thyroid hormones cause a marked stimulation of the synthesis of Na^+,K^+-ATPase in rat skeletal muscle (Asano *et al.*, 1976) as well as an increase in the concentration of [^{3}H]ouabain binding sites in skeletal muscle obtained from mice (Biron *et al.*, 1979), rats (Nørgaard *et al.*, 1983; Kjeldsen *et al.*, 1984a), and human subjects (Kjeldsen *et al.*, 1984c). In rats, however, K deficiency was not

associated with any significant change in plasma T_3 or T_4 hormone levels (Kjeldsen *et al.*, 1984a).

Since the reduction in [^{3}H]ouabain binding capacity seen during K deficiency might reflect a progressive occupation by an endogenous cardiac glycoside-like substance *in vivo*, [^{3}H]ouabain binding was also measured following a washout period of 120 min in Krebs–Ringer bicarbonate buffer. Neither this preincubation nor the prolongation of the equilibration with [^{3}H]ouabain for up to 360 min gave any change in the relative reduction in [^{3}H]ouabain binding capacity (Kjeldsen *et al.*, 1984a). Experiments in which [^{3}H]ouabain was injected intraperitoneally in rats showed that after 15 min, the soleus muscles had bound virtually the same amount of [^{3}H]ouabain as in the experiments performed *in vitro*. Again, K deficiency gave the same relative decrease in [^{3}H]ouabain binding (Clausen *et al.*, 1982). It seems unlikely, therefore, that the loss of [^{3}H]ouabain binding capacity is the result of increased occupancy of the receptors by an endogenous ouabain-like ligand.

There is evidence that down-regulation of [^{3}H]ouabain binding sites is not necessarily taking place in the entire pool of muscle cells, but may also be a localized response to an increased intracellular Na/K concentration ratio. Thus, denervation of individual muscles, which increases Na content and decreases K content, was found to reduce the concentration of [^{3}H]ouabain binding sites both in mice (Clausen *et al.*, 1981) and rats (Clausen *et al.*, 1983). It cannot be excluded, however, that this effect was the result of the concomitant decrease in muscle activity (Kjeldsen *et al.*, 1985b).

The importance of cellular K for maintenance of a normal Na^+,K^+-ATPase concentration in the sarcolemma is suggested by the observation that the synthesis of protein requires K (Ledbetter and Lubin, 1977). A general impairment of protein synthesis is known to develop during severe K deficiency (Rinehart *et al.*, 1968; Alexis *et al.*, 1971), and it was proposed that this might be of importance for the reduction in muscle [^{3}H]ouabain binding site concentration (Lamb *et al.*, 1982). However, even when the [^{3}H]ouabain binding site concentration was decreased by up to 41%, there was no change in plasma albumin concentration. As the liver maintains normal K contents during K deficiency, the synthesis of albumin, however, cannot be taken as a reliable indicator for overall protein synthesis.

When maintained on protein-deficient fodder with a protein content of 4 g/kg, i.e., around 2% of the protein content of normal fodder, for 4 or 8 weeks, mature rats showed a decrease in plasma albumin of 29% and 31%, respectively, as well as a complete cessation of weight gain. In the soleus muscle, the decrease in [^{3}H]ouabain binding site concentration was 10–12%, i.e., only a minor fraction of the 60% reduction developing during the administration of K-deficient fodder for the same interval of time (Kjeldsen *et al.*, 1984a). These observations indicate that the major part of the decrease in skeletal muscle [^{3}H]ouabain binding

site concentration is not the simple outcome of a general defect in protein synthesis, but rather reflects a selective interference with the control of the population of Na,K pumps.

VI. EFFECTS OF K DEFICIENCY ON Na,K TRANSPORT IN SKELETAL MUSCLE

In K-deficient rats, inactivation of individual muscles by tenotomy, plaster immobilization, or denervation led to a rapid, although incomplete recovery of the Na,K content (Akaike, 1979; Clausen *et al.*, 1983). This is likely to be the outcome of the decreased net K loss resulting from the cessation of action potentials. Thus, in spite of a considerable loss of Na,K pumps, resting muscles seem capable of partially restoring their intracellular Na,K contents even at the prevailing low extracellular K concentration. The Na,K pump defect is more evident in the working muscles, as seen from the higher intracellular Na/K concentration ratio.

Following the readministration of KCl to K-deficient rats, intracellular Na,K contents were normalized within a few hours, showing that at normal extracellular K concentrations *in vivo*, the Na,K pump defect is not readily detectable. On closer examination *in vitro*, however, such muscles isolated from K-depleted/K-repleted rats showed a reduced capacity for performing active Na,K transport (Kjeldsen *et al.*, 1985a). Soleus muscles obtained from young K-depleted/K-repleted rats showed a 35% decrease in ouabain suppressible ^{42}K uptake as compared to their age-matched controls. This is less than the relative decrease in [^{3}H]ouabain binding site concentration, which amounted to 61%. This discrepancy may in part be accounted for by the increase in intracellular Na, which could be detected in the muscles following equilibration *in vitro*. Thus, when this was corrected for, the relative decrease in ouabain-suppressible ^{42}K uptake amounted to 50%. The difficulties in detecting a functional Na,K pump defect under resting conditions *in vitro* are also related to the fact that the ouabain-suppressible rate of ^{42}K uptake in soleus muscles of 4-week-old rats amounts to only around 300 K ions per ouabain binding site per minute (Clausen and Hansen, 1974; Kjeldsen *et al.*, 1985a). This corresponds to 3% of the maximum capacity which can be calculated assuming full activation of all the available Na^+,K^+-ATPase units [12,000 ions/ouabain binding site/min at 30°C (Glynn, 1956; Sachs and Welt, 1967; Keynes and Ellory, 1969; Hoffman and Tosteson, 1971)]. On the basis of measurements performed using other intact muscles, a similar low utilization could be calculated. Thus, in rat diaphragm and mouse soleus, values of 7% and 4% were obtained under resting conditions *in vitro* (Creese, 1968; Biron *et al.*, 1979).

VII. EFFECTS OF K DEFICIENCY ON OTHER TYPES OF MUSCLE CELLS

Following 4 weeks on K-deficient fodder, the heart ventricles of mature rats showed around a 15% decrease in K content and a 20% increase in Na content (Nørgaard *et al.*, 1981, 1985c; Kjeldsen *et al.*, 1984a). Due to its low affinity for ouabain, the concentration of [^{3}H]ouabain binding sites in the rat myocardium could not be readily determined, but measurements of 3-*O*-MFPase activity showed a decrease of 14% in the K-deficient rats as compared to controls (Nørgaard *et al.*, 1985c). Measurements of [^{3}H]digoxin binding to the intact rat heart 1 hr after intravenous injection of [^{3}H]digoxin showed that the administration of K-deficient fodder for 2 weeks led to a reduction of 50% (Harrison and Brown, 1968). In contrast, other studies showed that K deficiency was associated with an increase of 130% in the Na^+,K^+-ATPase activity in membrane fractions isolated from guinea pig myocardium (Erdmann *et al.*, 1971). This discrepancy may be related to the fact that the purification of Na^+,K^+-ATPase activity from the myocardium only allows the recovery of a few percent of the total activity present in the starting material (for discussion, see Jones and Besch, 1984, and Nørgaard *et al.*, 1985b).

K deficiency is associated with paralysis of smooth muscle in most of the gastrointestinal tract, but no information is available on the Na,K contents. In resistance vessels of mature rats that had been K depleted for 4 weeks, the Na content was shown to increase, and the concentration of [^{3}H]ouabain binding sites was reduced by 37% (Aalkjaer *et al.*, 1985). Taken together, the evidence shows that during K deficiency, the K/Na concentration ratio as well as the concentration of [^{3}H]ouabain binding sites decrease in heart ventricular, smooth, as well as skeletal muscle cells.

VIII. IMPLICATIONS OF REDUCED Na,K PUMP CAPACITY FOR MUSCLE Na,K HOMEOSTASIS AND CLEARANCE OF Ca^{2+} AND H^+

The progressive decrease in the concentration of Na,K pumps with K deficiency leads to a reduced maximum capacity for active Na,K transport. This contributes to the maintenance of the high intracellular Na concentration. Although the remaining Na,K pumps will be activated by the high intracellular Na concentration, the low extracellular K concentration will be a limiting factor, and as long as there are no external supplies of K, the concentration gradient for Na across the plasma membrane will remain decreased. This will interfere with transport processes dependent on this gradient and its potential energy (Fig. 6). Thus, the

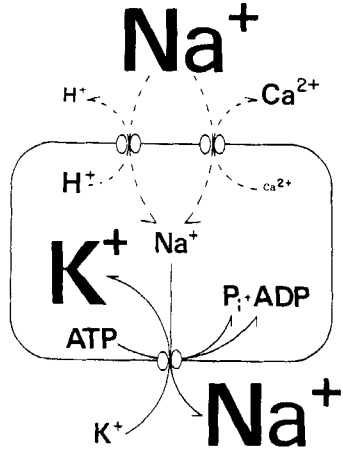

FIG. 6. Schematic diagram of the transport and distribution of cellular cations. The ouabain-suppressible Na,K transport is shown by solid lines whereas the Na^+/H^+ and Na^+/Ca^{2+} exchanges are shown by dashed lines. The size of the letters in this figure indicates the relative concentrations of Na and K outside and inside the cell membrane.

extrusion of H^+ ions via the Na^+/H^+ exchange mechanism will be impaired, leading to intracellular acidosis and extracellular alkalosis. Indeed, K deficiency is often associated with this particular acid–base disturbance (Welt *et al.*, 1960). Due to the effect of H^+ ions on renal K excretion, an increase in plasma pH (Burnell *et al.*, 1956; Adrogue and Madias, 1981) can be expected to aggravate the existing hypokalemia and further reduce the Na gradient-dependent H^+ extrusion from muscle cells.

Likewise, the clearance of cytoplasmic Ca^{2+} via the Na^+/Ca^{2+} exchange mechanism will be reduced, favoring the intracellular accumulation of calcium. This mechanism may account for the observation that K deficiency is associated with increased muscle calcium content. As pointed out by Knochel (1982), the mitochondrial swelling and damage seen in K-deficient muscles may reflect calcium overloading.

Finally, the selective decrease in muscle K leads to a reduction in K release during exercise, with ensuing impairment of vasodilation and oxygenation of the working muscles (Knochel, 1982).

The skeletal muscles contain the largest single pool of K in the body. On the basis of the concentration of [^{3}H]ouabain binding sites in human skeletal muscles (Kjeldsen *et al.*, 1984c), it can be calculated that the maximum capacity for active transport of K into the total pool of muscle cells in an adult human subject amounts to 125 mmol/min. This value is calculated on the basis of an [^{3}H]ouabain binding site concentration of 280 pmol/g wet weight, assuming that the total skeletal muscle mass is 28 kg and that the maximum molecular activity of the

Na^+,K^+-ATPase is 16,000 K/min at 37°C (Glynn, 1956; Sachs and Welt, 1967; Keynes and Ellory, 1969; Hoffman and Tosteson, 1971). As the total K content in the extracellular phase is around 63 mmol, it is evident that, depending on the activity of the Na,K pump, the skeletal muscles may either flood the plasma with K or induce hypokalemia. Thus, the demand for a tight control of skeletal muscle Na^+,K^+-ATPase is obvious. In mature rats, the maximum capacity for active K transport into the skeletal muscle pool is 400 μmol/min and total extracellular K is 180 μmol.

During electostimulation of muscles, the intracellular Na/K concentration ratio increases (Sreter, 1963), indicating that the capacity for K reaccumulation is exceeded. In agreement with these results, maximal exercise in dogs increases the interstitial K concentration to 9 mmol/liter (Hnik *et al.*, 1976), and in human subjects an increase in arterial blood K concentration by 3 mmol/liter has been reported during exercise (Hermansen *et al.*, 1984). The effect of exercise on the plasma K concentration has been shown to be more pronounced if the stimulating effect of catecholamines on the Na,K pump is blocked by adrenoceptor blocking agents (Carlsson *et al.*, 1978). Furthermore, the inhibition of the $Na,^+,K^+$-ATPase by ouabain *in vivo* showed an increase in plasma K in rat (Clausen *et al.*, 1982), and digitalis intoxication in human subjects is associated with hyperkalemia (Smith and Willerson, 1971; Citrin *et al.*, 1972). The relationship between a decrease in [^{3}H]ouabain binding site capacity and plasma K during exercise remains to be investigated. This should be done after K repletion in order to ensure comparability with respect to muscle Na,K content.

IX. IMPLICATIONS OF REDUCED Na,K PUMP CAPACITY FOR MUSCLE PROTEIN SYNTHESIS AND CELLULAR INTEGRITY

K deficiency is associated with retardation of growth (Cannon *et al.*, 1952) and poor utilization of dietary nitrogen sources. Even modest K deficiency may increase the minimum protein requirements to maintain nitrogen balance (Sapir *et al.*, 1976). In view of the selective increase in intracellular Na, this may in part be due to impaired accumulation of amino acids via Na gradient-dependent transport systems in the sarcolemma. Furthermore, studies with isolated cells have shown that K is required for protein synthesis (Ledbetter and Lubin, 1977) and that K deficiency leads to inhibition of muscle protein synthesis in rats (Alexis *et al.*, 1971).

Ca^{2+} activates muscle proteases (Dayton *et al.*, 1976), and the above-mentioned inhibition of cellular Ca^{2+} clearance may contribute to reduced net protein synthesis. Thus, the increase in intracellular concentration of amino acids with K depletion (Sanslone *et al.*, 1970) can be the net outcome of a decrease in

accumulation and an impaired utilization of amino acids together with an increased protein degradation. In addition, Ca^{2+} stimulates phospholipases, favoring the breakdown of membrane structures.

Taken together, these changes in muscle Na,K homeostasis, Ca^{2+} and H^+ clearance, protein synthesis, and maintenance of cellular integrity with K deficiency may ultimately cause overall celular damage and account for the local necrosis found in the muscles (Knochel and Schlein, 1972) and the myocardium (Kim and Harrison, 1972) of the K-deficient organism.

X. IMPLICATIONS OF REDUCED SKELETAL MUSCLE [^{3}H]OUABAIN BINDING CAPACITY FOR DIGITALIS GLYCOSIDE DISTRIBUTION

The skeletal muscles contain the largest single pool of digitalis receptors in the body. Measurements in mature rats (Nørgaard *et al.*, 1985c), guinea pigs (Kjeldsen *et al.*, 1985c), and human subjects (Kjeldsen *et al.*, 1985d) show that this pool is, respectively, 12, 36, and 40 times larger than the total number of digitalis receptors in the heart. In rats, the affinity of [^{3}H]ouabain for its receptor was lower in the heart than in the skeletal muscles (Nørgaard *et al.*, 1985c; Kjeldsen *et al.*, 1985c). In guinea pigs, no major difference in affinity of [^{3}H]ouabain for its receptor was found between these two tissues (Kjeldsen *et al.*, 1985c). Furthermore, in human subjects, we have recently found no major difference between heart and skeletal muscle in [^{3}H]ouabain affinity for its receptor. Therefore, following the administration of digitalis, by far the major part will be bound to the skeletal muscles. Thus, in the acute phase of a digitalization, a reduction in the muscle binding capacity induced by K deficiency will favor the redistribution of digitalis toward the plasma and make a larger quantity available for binding to the heart. This view is supported by the observation that following the injection of a single dose of [^{3}H]ouabain, the acute rise in plasma ^{3}H activity was 77% larger in K deficient rats than in age-matched controls receiving the same dose per kilogram of body weight (Clausen *et al.*, 1982). Furthermore, up to 24 hr after the intravenous injection of [^{3}H]digoxin in rats the activity in plasma was higher in K-depleted than in normal rats (Harrison and Brown, 1968).

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Chapter 19

Relationship between Cell Potassium and Hydrogen Ion in Muscle

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I. INTRODUCTION

Early experiments by Berliner and his colleagues in the kidney provided data which they used to hypothesize that potassium and hydrogen ions shared a distal renal secretory mechanism linked to sodium reabsorption (Berliner *et al.*, 1954). Subsequent work, presented in deatil elsewhere in this volume, has more completely clarified the relationships existing between renal potassium and hydrogen ion excretion and secretion. Analogous to the situation in kidney, there are close relationships between potassium and hydrogen ion activity and ionic movement in skeletal muscle. Alterations in extracellular acidity affect muscle potassium content, while changes in body and/or serum potassium levels alter muscle cell acidity and buffering capacity (Adler and Fraley, 1977). Most investigations of altered potassium states have focused on changes in extracellular acidity despite the now well-recognized fact that changes in extracellular pH do not necessarily reflect changes in intracellular pH (Roos and Boron, 1981). Since enzymes and chemical reactions exhibit pH optima and as most metabolic reactions occur inside rather than outside of cells, it is important to determine precisely how potassium affects intracellular acidity. Unfortunately, the exact relationship between muscle cell pH and potassium is not clear. One major reason for this ambiguity is the difficulty in defining and measuring cell pH. First, therefore, the methods available for measuring muscle cell pH and the limitations of these methods will be described.

II. TECHNIQUES FOR MEASURING CELL pH

A few excellent reviews describe the various techniques used to measure cell pH. For a more detailed analysis than that given below, the interested reader should refer to the recent review by Roos and Boron (1981). Some general principles require enumeration. First, the muscle cell, like other cells, is heterogeneous in regard to its internal pH. Cytoplasmic and mitochondrial pH, for example, may differ by more than 0.5 pH units under physiological conditions (Adler *et al.*, 1984), and external conditions alter the degree of intracellular pH heterogeneity found in muscle (Adler *et al.*, 1974a). A second general consideration is that pH differences may and probably do occur across the surface of protein molecules. Different techniques of cell pH measurement may therefore give a different value for cell pH, depending upon the particular portion or portions of the cell being examined. Even a single technique when used to measure cell pH, such as the microelectrode, detects significant pH differences between the surface pH and the deep or bulk cytoplasmic pH. This might be due to the ionic diffusion of molecules such as carbon dioxide or lactate (de-Hemptinne and Huguenin, 1984). A third general consideration regarding cell pH measurement is the difference between equilibrium cell pH values and changes in hydrogen ion or hydroxyl ion transport. Cell pH may be altered acutely by raising carbon dioxide tension, but the equilibrium or steady-state pH may be unchanged due to physical chemical buffering, changes in metabolic acid production, organellar H^+ translocation, and active transport mechanisms which restore cell pH toward normal (Roos and Boron, 1981). Changes in cellular buffering and/or transport of hydrogen and hydroxyl ions could be linked to changes in cellular metabolism. Thus, the equilibrium cell pH value alone is probably insufficient for describing the effect of various pathophysiological conditions, such as potassium depletion or acute potassium loading, upon muscle cell pH. This point will be examined subsequently in more detail. A final general consideration is that the method of measurement may itself alter the cell pH. Microelectrodes, for example, could induce cellular injury, and weak acids for bases might influence cellular metabolism and cell pH. Keeping these general principles in mind, the different techniques for measuring cell pH and their advantages and limitations, particularly as they apply to the measurement of muscle cell pH, are delineated below.

A. Tissue Homogenates and Lysates

This is an old method first used in 1912 for measuring red blood cell pH (Michaelis and Davidoff, 1912). This seemingly simple and direct method suffers from the fact that cells are disrupted, intracellular and extracellular fluids are

mixed, and organelles are disrupted by the homogenization or lysing process. Its greatest usefulness is for measuring the pH within erythrocytes and possibly in sea urchin eggs. It can be used to measure physical chemical buffering capacity, but has limited usefulness for *in vivo* vertebrate muscle experiments.

B. Chemical Equilibria

This method utilizes a pH-dependent chemical reaction. The cell pH *at equilibrium* can be calculated from the concentration of the substances participating in the reaction and the pK value. Two reactions employed have been the creatine phosphokinase equilibrium and the lactate:pyruvate or NADH:NAD equilibrium. This method is severely limited because the pK inside the muscle is often unknown, the precise location and measurement of the various molecular species is difficult (e.g., free ADP in the creatine phosphokinase reaction), the establishment of equilibrium conditions is uncertain, and only a single measurement can be obtained. It has not been used, therefore, for muscle cell pH calculations in abnormal potassium states and has been superseded by the other methods outlined below.

C. Colorimetry and Fluorescent Probes

In this method, a pH-sensitive dye or fluorescent marker is introduced into the cell and the pH is determined from the change in color or fluorescence. Until now its limitations for muscle pH measurement have been enormous, so it has not been used significantly in this tissue except to study transient pH changes during contraction (MacDonald and Jöbsis, 1976). It has great utility, however, for measuring pH changes in phagocytizing cells, acid-secreting cells, membrane vesicles, storage granules, and mitochondria. In these cells and organelles, it has been employed with great effectiveness to study hydrogen ion transport.

D. Weak Acid or Base Distribution

This method and the following two methods are the major ones used to study muscle cell pH. It is also the single method which has contributed most of the data presently available about the effect of abnormal potassium states on muscle cell pH in mammals. The method is based on the assumption that upon dissociation of a weak acid or base, the undissociated form is permeant and able to pass freely across the cell membrane. The changed or dissociated form, however, is relatively impermeable. Thus, the concentration of the undissociated acid or base is equal on both sides of the membrane, while the dissociated or changed form is

distributed as a function of the pH on each side of the membrane (Waddell and Bates, 1969). The most common compounds used have been the acids DMO and carbonic acid (CO_2 method) and the weak bases ammonia and nicotine. The major advantage of the technique is the ease with which it can be used, particularly when combined with an isotope marker. It has been employed successfully in whole animals, isolated muscle tissues, and cell preparations. The disadvantages, enumerated well by Roos and Keifer (1982), include the ability to make only a single measurement, allowance of sufficient time for equilibration of the marker, and possible binding of the substance to cellular components which, in the case of a weak acid, would falsely elevate the calculated pH or falsely lower it in the case of a weak base. Other problems include metabolic transformation of the marker, difficulty in exactly measuring the extracellular space, assumptions regarding the pK of the acid or base inside the cell, and the precise interpretation of the calculated cell pH value obtained. As mentioned, there are different regions of pH within the cell. Adler *et al.* (1984) have shown that in rabbit, renal tubular cell calculation of cell pH from distribution of the weak acid DMO gives a value which is primarily the sum of cytoplasmic and mitochondrial pH. This shows that the cell pH value derived from weak acid or base distribution is niether a cytoplasmic nor an organelle pH alone. Mathematically, the following relationship clarifies this point:

$$pH_{acid} > pH_i > pH_{base}$$

In this expression, the values represent, respectively, the pH value calculated from distribution of a weak acid, the true mean intracellular pH, and the pH value calculated from the distribution of a weak base. Despite the limitations listed, the method is extremely useful, particularly in showing directional changes in cell pH when a perturbation such as hypokalemia or hyperkalemia is applied to the system. Newer applications of this particular method include a rapid washout technique which could assist in the examination of cell pH regulation (Roos and Baron, 1978).

E. Microelectrodes

This is the benchmark method or standard to which data obtained by other methods are often referred. It is a direct extension of the measurement of solution pH by pH-sensitive glass electrodes. New smaller electrodes have been designed to penetrate cells without causing serious injury. The major advantage of the microelectrode technique is its ability to continuously and rapidly record cell pH within a single cell. When the technique is carefully performed, it gives a stable measurement associated with little injury current. A major problem, already alluded to, is the difficulty in constructing electrodes small enough to prevent

injury, yet sufficiently stable to minimize electrical artifacts. Most often the cell must be pierced by two electrodes (glass and reference), increasing the possibility of injury. Another disadvantage is that the pH recorded almost certainly measures only the bulk cytoplasmic component, and no information regarding organelle pH is obtained. The method, despite the difficulties and limitations, has been used increasingly during the past 10 years to determine the fundamental ways in which cells regulate their internal pH. Much information on vertebrate muscle pH regulation has been obtained using microelectrodes in mouse soleus muscle (Aickin and Thomas, 1977).

F. Phosphorus Nuclear Magnetic Resonance (^{31}P-NMR)

This is the most recent method for studying cell pH in vertebrates, particularly in man. It is being employed extensively for the study of muscle cell pH and muscle bioenergetics (Gadian *et al.*, 1981). As it is the least familiar of the methods listed for measuring cell pH and as data obtained using this method in potassium-deficient rat brain will be described later in the chapter, this method will be described in some detail and an example shown. The method depends on the fact that many nuclei, among them ^{31}P, possess a magnetic moment in a directionally applied static magnetic field. The intrinsic magnetic properties of the nuclei may be examined by observing the characteristic frequencies at which the nuclei absorb and then emit radiofrequency radiation. The magnetic properties of the ^{31}P nuclei vary depending on the chemical environment. The latter alters the frequency of the radiation emitted allowing differentiation among the various ^{31}P molecular species. This frequency of the inorganic phosphate signal is sensitive to changes in pH within the physiological range so that determination of its frequency with reference to internal or external standards permits the measurement of intracellular pH. In muscle, heart, and brain, there is a simple internal standard, phosphocreatine, whose frequency is not affected by pH. Thus, the distance between inorganic phosphate and phosphocreatine on the frequency spectrum is a measure of cell pH. The major advantages of ^{31}P-NMR are that it is noninvasive and nondestructive, it allows sequential and continuous measurement of intracellular pH over a prolonged time period, it is rapid, usually requiring only 1–2 min for a cell pH determination *in vivo*, and it simultaneously measures other phosphorylated compounds such as ATP, phosphocreatine, and sugar phosphate. There are also disadvantages and limitations of the technique. It is quite complex and expensive, requiring special equipment and highly trained personnel familiar with the methodological problems. Also, it has an inherent degree of insensitivity so that cell pH can be measured at best only to an accuracy of about 0.05 pH units. The inorganic phosphate concentration on which the measurement depends may be too low to provide good spectral resolution, thus

requiring longer periods of collection and decreased time resolution. In addition, the inorganic phosphorus signal probably reflects only cytoplasmic pH (Adler *et al.*, 1984). An example of ^{31}P-NMR obtained *in vivo* from rat gluteus muscle is given in Fig. 1. The distance between the inorganic phosphate and phosphocreatine peaks under control conditions (top) was 4.97, which is equivalent to a pH of 7.11. When the animal was ventilated with a 20% CO_2 gas mixture, thereby inducing a respiratory acidosis (bottom), the inorganic phosphate–phosphocreatine distance decreased to 4.58, corresponding to an intracellular pH of 6.81. The ^{31}P-NMR method holds great promise for the study of cellular pH regulation in abnormal conditions and for determining the relationship between cell pH and metabolism *in vivo*.

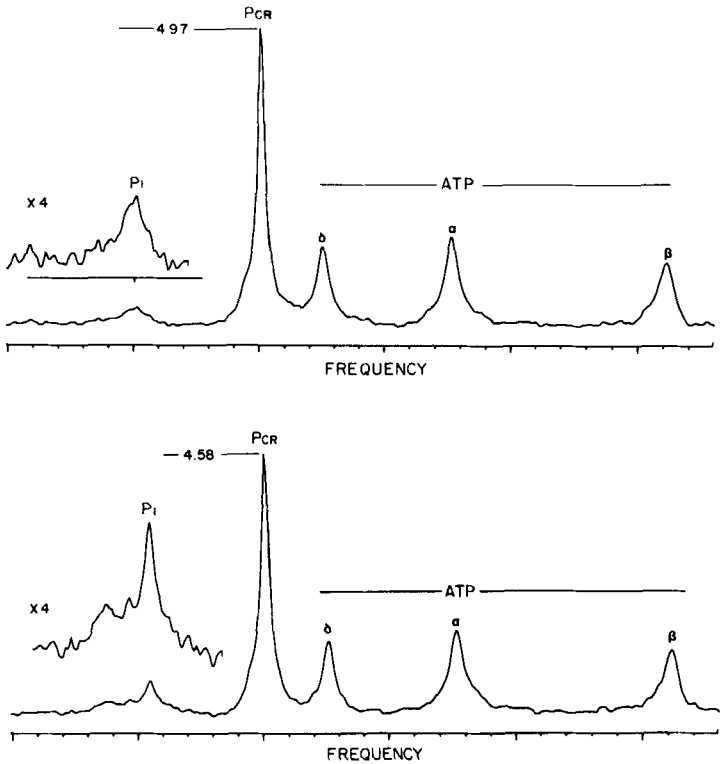

FIG. 1. Frequency spectrum of rat gluteal muscle obtained by ^{31}P-NMR at 121.5 mHz. The top half shows spectrum obtained under control conditions with rat ventilated with O_2–N_2O. The bottom half shows the effect of ventilation with 20% CO_2 balance O_2–N_2O. The P_i (inorganic phosphate)–P_{Cr} (phosphocreatine) distance is a measure of cell pH.

III. MUSCLE CELL pH REGULATION AND SOME POSSIBLE ROLES FOR POTASSIUM IN THIS REGULATION

That potassium and cell pH are somehow interrelated is a very old concept. As far back as 1934, Fenn and Cobb (1934) reported that lowering the pH of a saline bathing medium by raising the CO_2 tension caused potassium to move out of skeletal muscle. However, when the medium used was blood instead of saline, potassium moved in the opposite direction. The authors postulated that muscle tissue is better buffered than saline so that the fall in cell pH was less than the fall in the pH of the saline solution. The converse result occurring when blood was the bathing medium made the authors conclude blood was better buffered than muscle. This led to the concept that there was a mechanism, possibly a Donnan equilibrium, which maintained rough equality between the intracellular and extracellular potassium and hydrogen ion ratios (Waddell and Bates, 1969). A great many observations have confirmed the fact that in acidosis skeletal muscle potassium concentration falls and the extracellular potassium concentration rises (Burnell *et al.*, 1956). It should be noted, however, that infusion of organic acids such as lactic or β-hydroxybutyric acid does not release skeletal muscle potassium as does HCl or NH_4Cl infusion (Oster *et al.*, 1978). Wadell and Bates (1969) reviewed the then available data in rat, cat, dog, and man concerning the relationship between cell pH and potassium distribution across skeletal muscle. There appeared to be a linear relationship between the logarithmic change in the intracellular to extracellular ratios of the two ions. Of great interest was a similar demonstrable relationship of changes in the transcellular sodium distribution, suggesting there might be common factors responsible for regulating the intracellular and extracellular ratio of all three ions. The changes in the sodium gradient in the various experimental situations, however, were found to be at least fivefold greater than the changes in either the hydrogen ion or potassium ion ratios. Alterations in the binding of sodium or errors in the measurement of the intracellular sodium concentration, a difficult determination at any time due to the mainly extracellular location of the ion, were thought to account for the apparent disparity.

Further experimentation, however, has refuted the hypothesis that the ratios of H^+_{int}/H^+_{ext} and K^+_{int}/K^+_{ext} are linked and move together quantitatively. Rather, the relationship between the two ions is more complex than originally proposed. This conclusion derives from a host of observations consonant with the present theory of how muscle cell pH is regulated. The first observation is that when the data upon which the original hypothesis was erected are examined closely, they exhibit a great deal of scatter. In rats and dogs, a 0.15 unit change in the logarithmic potassium ion ratio occurs over a pH gradient change which

varies between 0.10 and 0.50 (Waddell and Bates, 1969). The second observation relates to the fact that there are sharp differences between individual tissues in regard to their behavior during acid–base perturbations. Thus, despite similar decreases in skeletal muscle and cardiac muscle cell pH during respiratory acidosis, potassium moves out of skeletal muscle but moves into the cardiac muscle (Young *et al.*, 1954). In cardiac muscle, therefore, neither passive Donnan forces nor ionic ratios can explain the observed results. Similarly, data presented later in this chapter show that rat brain cell pH is lower in potassium depletion despite absence of a significant dhange in brain potassium content. The third series of observations refer to experiments in which isohydric changes in extracellular conditions alter the potassium gradients across the muscle without altering the pH gradient. Kim and Brown (1968), using the weak acid DMO to measure cell pH, showed that volume expansion in nephrectomized dogs by either saline or mannitol solutions lowered the inintracellular to extracellular potassium ratios, but did not affect the pH gradient. Similarly, Makoff *et al.* (1971) showed that the infusion of hypertonic saline or mannitol into nephrectomized dogs raised the whole body pH of these animals (essentially skeletal muscle pH) at a time when extracellular pH fell. As extracellular potassium rose, the logarithmic ratios of both K^+_{int}/K^+_{ext} and H^+_{int}/H^+_{ext} fell. Yet, when variable amounts of sodium bicarbonate were added to the infusate to regulate changes in the extracellular pH, the changes in the transcellular potassium and hydrogen ion ratios were no longer the same. In each of 14 separate experiments, the H^+_{int}/H^+_{ext} fell, but in 10 of the 14 experiments, the K^+_{int}/K^+_{ext} rose, a clear example of the dissociation between these two ionic ratios. Further evidence for the dissocation of these ionic ratios in muscle has been obtained *in vitro*. Adler *et al.* (1972), using an *in vitro* intact rat diaphragm muscle preparation, measured cell pH from distribution of the weak acid DMO or the weak base nicotine. The intracellular to extracellular potassium ion ratios were altered by acutely depleting the muscle *in vitro* by incubation in a potassium-free medium. At an external pH of 7.40, intracellular pH was unaffected by this maneuver despite a 10-fold increase in the intracellular to extracellular potassium ratio.

In both rat (Fraley and Adler, 1976) and man (Fraley and Adler, 1977), isohydric elevation of the blood bicarbonate concentration causes potassium to move into cells, presumably the muscle. This maneuver should raise cell hydrogen ion activity as increasing the CO_2 tension at a constant external pH causes skeletal muscle pH to fall (Adler *et al.*, 1965). The changes in extracellular potassium and hence the potassium intracellular to extracellular ratio, however, are much greater in magnitude than the expected changes in the pH gradient across the muscle. From all these data, it is apparent that the relationship between potassium and hydrogen ions in skeletal muscle is complex and not determined by a Donnan distribution. To understand these relationships, it is now necessary to review briefly the present working hypothesis of how cells, presum-

ably including msucle, regulate internal acidity. A detailed and full analysis of cell pH regulation is given by Roos and Boron (1981) in their excellent review.

The pH of most cells, including skeletal muscle, is maintained outside of thermodynamic equilibrium. At an external pH of 7.40 and assuming a muscle transmembrane potential (V_M) of 80–90 mV, cell interior negative, skeletal muscle pH should lie between 5.90 and 6.10. Measurement of muscle cell pH, however, using microelectrodes, weak acid distribution, or ^{31}P-NMR, shows that muscle cell pH lies between 6.90 and 7.00 or approximately an entire pH unit higher than predicted from thermodynamic considerations. Thus, there must be active efflux of hydrogen ions. It appears that hydrogen ion regulation in most cells, including muscle cells, best fits a pump leak system with physical chemical buffering and acid production of the type shown in Fig. 2. Hydrogen ions are constantly being added to the cell by two mechanisms. The first of these is the passive flux of hydrogen ions across the membrane down its electrochemical gradient (or hydroxyl and/or bicarbonate ions moving in the opposite direction). The second mechanism for cellular hydrogen ion addition is through metabolic acid production. In the equilibrium or steady-state condition, this acid loading of the cell (J_L) will be balanced by the rate of acid extrusion (J_e). Raising J_L by various means, such as raising CO_2 tension, will decrease cell pH. Decreases in cell pH stimulate active H^+ transport and increase J_e (Roos and Boron, 1981). Thus, alterations in any of the components of the system could affect steady-state cell pH and active hydrogen ion transport. In mouse soleus muscle (Aickin and Thomas, 1977), chick skeletal muscle cells (Visne *et al.*, 1984), and a variety of

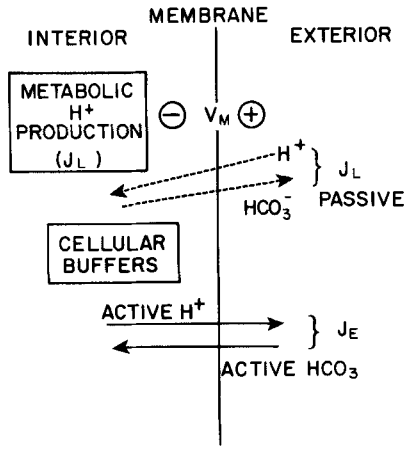

FIG. 2. Diagrammatic representation of cell pH regulation in skeletal muscle. V_M is the transmembrane potential, J_L is the acid loading of the cell, while J_e represents acid extrusion. Cellular buffers refer to all buffers available for physicochemical buffering.

invertebrate tissues (Roos and Boron, 1981), an important mechanism for acid extrusion is through amiloride-sensitive exchange of external sodium ions for cellular hydrogen ions. In mouse soleus muscle, this mechanism appears to account for 70% of the tissue's active acid extruding capacity (Aickin and Thomas, 1977). The sodium pump which exchanges intracellular sodium for extracellular potassium does not appear to be involved in internal hydrogen ion regulation. Blockade of the pump with ouabain in rat diaphragm muscle (Adler, 1975), sheep Purkinje fibers (Ellis and Thomas, 1976), or mouse soleus muscle (Aickin and Thomas, 1977) does not alter cell pH. Indeed, in the latter preparation, removal of extracellular potassium does not inhibit acid extrusion. There is evidence suggesting that a chloride–bicarbonate exchange mechanism may also be important for regulating hydrogen ion extrusion in muscle and that cyclic AMP also may be involved in cell pH regulation (Roos and Boron, 1981). Interestingly, β-adrenergic blockade prevents K^+ uptake by muscle and interferes with muscle buffering and acid extrusion (Clancy *et al.*, 1976).

From the formulation shown in Fig. 2, it is obvious that a number of factors might affect and regulate steady-state cell pH. A rise in extracellular bicarbonate concentration or extracellular pH would tend to raise cell pH by increasing hydrogen ion extrusion and/or decreasing bicarbonate efflux (Roos and Boron, 1981). Moreover, raising the external sodium concentration could raise cell pH while a decrease in the external chloride concentration might also raise cell pH by affecting the sodium–hydrogen ion and chloride–bicarbonate exchange mechanisms. How, then, might laterations in body potassium balance influence cell pH regulation and muscle cell pH? Three distinct possibilities emerge. First, alterations in potassium could change the transmembrane potential of the muscle. Khuri *et al.* (1976) showed that in states of potassium depletion V_m rose, while after potassium administration, there was a fall in V_m. This would, respectively, increase and decrease passive hydrogen ion influx. Unopposed by any buffering mechanisms, potassium depletion would cause an intracellular acidosis, while potassium administration would cause an intracellular alkalosis. Second, changes in cellular potassium could alter cellular metabolic acid production. It is known that potassium depletion induces metabolic changes in kidney tissue (Tannen and McGill, 1976) and in skeletal muscle (Adler, 1974b). However, the exact mechanism by which these metabolic changes are brought about is unknown. In the muscle, it appears to be related to changes in pH, but recent data suggest that in the kidney potassium depletion might not alter steady-state pH. Moreover, total body potassium depletion alters the potassium content of tissues by sharply different amounts so that the effect of potassium depletion in different tissues could be quite variable. Third, changes in potassium balance might alter external conditions, thereby influencing cellular hydrogen ion extrusion (J_e). As stated earlier, in most states of potassium depletion there is an extracellular metabolic alkalosis. Extracellular pH and bicarbonate are elevated. Increases in

these quantities would enhance acid extrusion (or decrease bicarbonate efflux), which could ameliorate the effect of the increased transmembrane potential. The latter, as mentioned earlier, would increase acid loading of the cell (J_L) by increasing passive hydrogen ion entry. From this brief summary, it is obvious that the effect of alterations of potassium balance on cell hydrogen ion concentration is complex and that the precise mechanisms controlling and regulating these two ions are still not fully determined. It is also obvious that the original hypothesis of Donnan forces regulating muscle K^+ and H^+ is incorrect. Finally, recognizing the heterogeneous nature of cell pH and possibly cellular potassium distribution, better methods for measuring cell pH and free intracellular potassium, sodium, and chloride concentrations in specific areas of the cell will be necessary before potassium and intracellular hydrogen ion relationships can be fully defined.

IV. EFFECT OF POTASSIUM DEPLETION UPON INTRACELLULAR pH

States of potassium depletion in man are usually associated with metabolic alkalosis (Jacobson and Seldin, 1983) unless there is concomitant bicarbonate loss in the stool or urine. This alkalosis has been extensively investigated and attributed to potassium depletion per se, accompanying chloride depletion, volume contraction, or a combination of all three. Despite controversy over the induction and maintenance of the alkalosis, it is generally agreed that potassium depletion reduces muscle pH. Cooke *et al.* (1952) showed that when potassium-depleted rats were potassium repleted, the muscle gained three potassium ions for each two sodium ions lost. The difference they felt was due to hydrogen ions which had entered the muscle during depletion and left it during repletion. This indirect evidence of intracellular acidosis, however, can be explained without invoking reduced cell pH. Basic amino acids, such as lysine, accumulate in muscle during potassium depletion (Eckel *et al.*, 1959) and could account for the sodium–potassium difference. Altered cell binding, variations in cellular anions, or changes in metabolic acid production could also account for the observation. Subsequent work, however, confirmed the presence of cellular acidosis. *In vivo* measurements of muscle pH in potassium-depleted rats using various methods (Adler *et al.*, 1972; Khuri *et al.*, 1976; Gardner *et al.*, 1952) all show reduced intracellular pH. Data obtained in dogs support this conclusion. Wilson and Simmons (1970), using DMO to measure cell pH, found reduced muscle cell pH in potassium and chloride-depleted dogs. Interestingly, the sum of intracellular sodium and potassium in their animals was increased rather than decreased. Many explanations, including altered binding and changes in anionic sites, were advanced to explain the latter finding, but it remains unexplained. Recent work

in potassium-depleted rats using electron microprobe electrolyte analysis (Beck *et al.*, 1982) found the sum of sodium and potassium in potassium-depleted kidney and hepatic tissue reduced and the gain in sodium only one-fifth the potassium loss. Different tissues thus appear to respond differently to potassium depletion, but usually the sodium and potassium changes are unequal. Grantham and Schloerb (1965) also found muscle cell pH reduced in potassium-depleted dogs. By contrast, Burnell and Dawborn (1970) found a normal muscle pH in potassium-depleted dogs. However, blood pH was elevated, so the transmembrane pH gradient must have been increased. Consistent with other data (Adler and Fraley, 1977), they showed decreased muscle buffering capacity in the potassium-depleted dogs. Subsequent work from Burnell's laboratory (Larsen and Burnell, 1978) also showed a reduced buffer value of skeletal muscle in potassium-depleted dogs. There are few studies of muscle cell pH in potassium-depleted man, and direct cell pH measurements are lacking. Two recent studies, however, present provocative data. Jones *et al.* (1982) induced potassium depletion in normal men by dietary deprivation. Plasma potassium fell from control values of 4.0 mEq/liter to 2.9 mEq/liter, with a cumulative body potassium loss of 300 mEq. Plasma bicarbonate and pH values rose slightly, but significantly. Since urinary net acid excretion fell, the data suggest an extrarenal mechanism for disposal of the retained hydrogen ions and for generation of the alkalosis. Thus, the data are consistent with hydrogen ion movement into the largest available buffer pool, skeletal muscle. An alternative explanation is that potassium depletion interfered with metabolic acid production, thus accounting for the reduced acid excretion. As the authors point out, this is unlikely, since much larger changes in endogenous acid production in man (Lennon *et al.*, 1962) or rat (Harris *et al.*, 1984) do not alter plasma acid–base balance. The second human study, reported only in abstract (Redaelli *et al.* 1983), examined the effect of altering dialysate potassium concentration on bicarbonate removal from the dialysate. When a 2 mEq/liter potassium dialysate was used, 242 mEq of bicarbonate were added to the body, of which 185 mEq (76%) disappeared from the extracellular space presumably consumed by cellular hydrogen ions. Dialysis with a potassium-free solution added only 177 mEq of bicarbonate, and only 78 mEq (44%) disappeared from the extracellular space. The authors suggest that dialysis with the potassium-free dialysate lowered the plasma potassium and increased the transmembrane potential. As shown in Fig. 2, increased V_m would increase passive H^+ influx, which could reduce cell pH and cellular buffering capacity.

Except for a single study (Miller *et al.*, 1963) whose apparent conflicting results can be explained, *in vitro* experiments confirm the *in vivo* studies. In addition to the muscle homogenate data, which show a reduced muscle buffer capacity in potassium depletion, Adler *et al.* (1972), using the weak acid DMO or the weak base nicotine, measured cell pH in isolated rat diaphragms depleted

of potassium *in vitro* (Fig. 3). Extracellular pH was altered at a constant external bicarbonate concentration by varying carbon dioxide tension. At an external pH of 7.40, muscle pH was only slightly lower in the depleted muscle, but at alkaline and particularly acid external pH values, cell pH was significantly lower in the potassium-depleted diaphragms. These data explain the results of Miller *et al.* (1963), who incubated diaphragms *in vitro* at a single external pH value of 7.40 and found no significant change in cell pH. *In vivo,* potassium depletion is commonly associated with elevated CO_2 tension, a condition in which, as shown in Fig. 3, potassium-depleted muscle pH is reduced.

Before examining the effect of potassium depletion on other tissues, it is necessary to briefly review if changes in muscle cell pH in potassium depletion are due to the potassium deficiency per se or to the accompanying chloride deficiency. Many naturally occurring metabolic alkaloses are correctable by saline infusion without correction of the potassium deficit. Nevertheless, a large

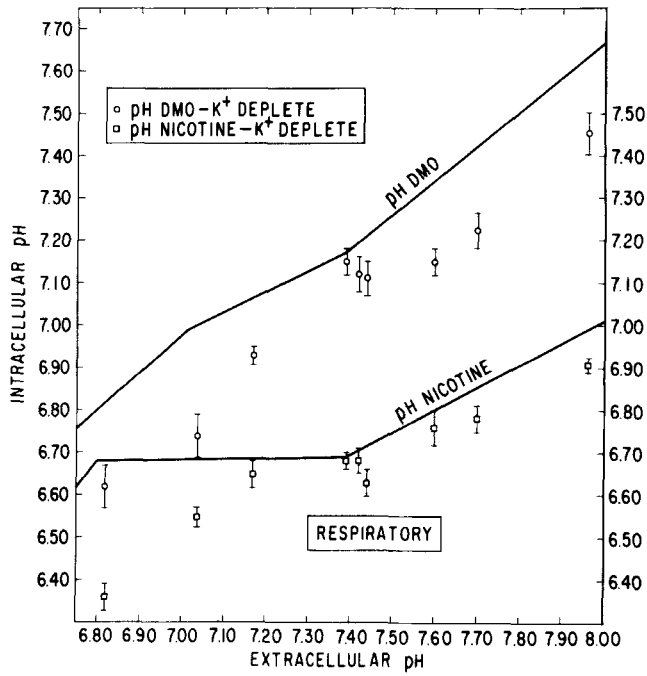

FIG. 3. Effect of potassium depletion on cell pH compared to that of non-potassium-depleted tissue as a function of varying extracellular pH by alterations in bath carbon dioxide tension at a constant bicarbonate concentration. The solid lines indicate the relationship previously reported for non-potassium-depleted 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 *et al.* (1972), with permission of the authors.]

number of metabolic alkaloses, particularly in mineralocorticoid excess syndromes, are chloride resistant, requiring potassium repletion to correct the extracellular acid–base disorder (Jacobson and Seldin, 1983). Despite the possibility of a chloride–bicarbonate exchange mechanism, most data suggest that intracellular acidosis of potassium depletion is due to the potassium, not the chloride ion. Khuri *et al.* (1976) measured muscle pH in chronically chloride-depleted rats. Despite mild metabolic alkalosis, muscle cell pH was normal. In a study of potassium and chloride-depleted dogs (Wilson and Simmons, 1970), extracellular metabolic alkalosis was corrected by chloride replacement, but intracellular acidosis was unaltered. Administration of chloride ions to potassium chloride-depleted rats in other experiments partially corrected the extracellular metabolic alkalosis, but urinary ammonia excretion increased and urinary citrate excretion decreased (Adler *et al.*, 1974c); these are results normally found in acidosis. In the human experiments of Jones *et al.* (1982), dietary potassium depletion decreased chloride excretion. Despite the absence of chloride deficiency, there appeared to be net retention of hydrogen ions intracellularly. Similarly, in the dialysis experiments cited earlier (Redaelli *et al.*, 1983), the reduced bicarbonate transfer was unrelated to chloride ions. Further confirmation of the primary role of potassium in altering muscle cell pH is found *in vitro* where adequate chloride in the incubation medium does not prevent a potassium depletion-induced reduction in cell pH (Adler *et al.*, 1972). One study, however, does suggest that chloride may influence muscle cell pH in potassium depletion. Burnell and Dawborn (1970) gave chloride to potassium chloride-depleted dogs and restored both extracellular and intracellular acid–base conditions to normal. Despite this study and the continued controversy regarding the relative roles of potassium, chloride, and extracellular volume in the regulation of renal bicarbonate reabsorption and hydrogen ion excretion (Jacobson and Seldin, 1983), it seems clear that potassium depletion per se does reduce muscle cell pH and lower its buffering capacity.

Potassium depletion appears to affect the pH of other tissues. Despite extracellular metabolic alkalosis, renal ammonia and citrate metabolism are altered in a fashion found in acidosis, suggesting the simultaneous presence of renal tubular cell acidosis and extracellular alkalosis (Huth *et al.*, 1959; Simpson, 1983). Direct microelectrode measurements in the proximal tubule cells of potassium-depleted rats show hyperpolarization and reduced potassium activity (Cemerikic *et al.*, 1982). Cell pH was not measured directly, but the increase in transmembrane potential would increase passive bicarbonate efflux or hydrogen ion influx. Cardiac muscle buffering capacity also is reduced by potassium depletion (Larsen and Burnell, 1978), but cardiac muscle is better buffered than skeletal muscle, so the intracellular pH of cardiac muscle has not been shown to be reduced in potassium deficiency (Cameron and Hall, 1975). Some unpublished work by the author of this article, obtained in collaboration with C. Ho and V.

Simplaceanu in rat brain, helps clearify the effect of potassium depletion on cell pH. Using ^{31}P-NMR, brain cell pH was determined in chronically potassium-depleted (KD) or potassium-repleted (KR) rats. Muscle cell potassium was reduced by ~40% in KD, but brain potassium content was identical in the two groups. After a control period, rats were ventilated for 30 min with a high (20%) CO_2 gas mixture to acid load the cells. Rats were then allowed to recover by ventilating them with the original zero CO_2 gas mixture. The transcellular pH difference (blood pH minus brain pH) was always greater during control, experimental, and recovery periods in KD versus KR rats. Nevertheless, the time course of buffering during high CO_2 exposure showed slightly more rapid buffering and achievement of equilibrium cell pH in KD rats. The results are best interpreted according to the concepts shown in Fig. 2. We believe potassium depletion hyperpolarized the cell membrane and increased passive hydrogen ion influx, thereby increasing the transcellular pH gradient. This H^+ influx presumably stimulated active hydrogen ion extrusion (Roos and Boron, 1981), accounting for the tissue's more rapid buffering response to elevated CO_2. Certainly, active H^+ transport was not reduced by K depletion.

All the foregoing experiments show that the absolute level of cell pH can be normal or reduced in potassium depletion, depending upon the extent of active hydrogen ion transport, physical chemical buffering, and metabolic acid production. Further experiments performed in potassium-depleted animals using ^{31}P-NMR or other methods should help clarify whether active hydrogen ion transport is affected in tissues such as muscle, whose potassium content is drastically lowered, compared to brain, where potassium content is essentially unaltered. Interestingly, Sanslone and Muntwyler (1967) could not relate the degree of intracellular acidosis to the degree of potassium deficiency in skeletal muscle. Also, Larsen and Burnell (1978) showed that cardiac muscle buffering was reduced in potassium depletion in the absence of significant potassium loss. In summary, the data suggest that the pH and buffering capacity of most tissues, especially skeletal muscle, is reduced in potassium depletion. The degree of reduction, however, varies from tissue to tissue. Increased cellular acidity appears to be primarily caused by passive H^+ influx or bicarbonate efflux. Active hydrogen ion extrusion may be secondarily stimulated, so equilibrium cell pH will vary depending upon the particular tissue and the external acid–base conditions.

V. RELATIONSHIP BETWEEN HYPERKALEMIA AND MUSCLE CELL pH

As expected, the effect of acute hyperkalemia on muscle cell pH seems the reverse of that found in potassium deficiency. Administration of potassium or

rubidium chloride to normal or potassium-depleted rats causes a metabolic acidosis, and in normal rats this acidosis is associated with a transient rise in muscle cell pH (Adler and Fraley, 1977). Muscle cell pH returns to normal by 6 hr. In potassium and chloride-depleted dogs given potassium chloride, acutely skeletal muscle pH rose from 6.87 to 7.00 (Burnell and Dawborn, 1970), and potassium or rubidium chloride given to potassium chloride-depleted rats acutely raised muscle cell pH (Hudson and Relman, 1962). Dua *et al.* (1975) gave KCl intravenously to normal dogs and caused an extracellular metabolic acidosis, an intracellular alkalosis, and bicarbonaturia. *In vitro* data also show that potassium acutely increases muscle pH. Thus, incubation of rat diaphragms in elevated potassium media raises cell pH (Miller, *et al.*, 1963). Data on muscle pH in chronic hyperkalemia, however, are lacking. Khuri *et al.* (1976) chronically potassium loaded rats, but no plasma potassium values were given. Muscle cell pH showed an increase in cell bicarbonate concentration, but a normal cell pH. As these rats had respiratory acidosis, the elevated CO_2 tension alone could have caused the increased cell bicarbonate. Alternatively, a normal cell pH in the presence of an elevated CO_2 tension suggests increased hydrogen ion extrusion, decreased H^+ influx, or tissue buffering, since CO_2 elevation should lower cell pH. At this time, therefore, all that can confidently be stated is that acute hyperkalemia probably increases muscle cell pH. The mechanism presumably is through a reduction in the V_m and a decrease in passive H^+ influx. The effect of chronic hyperkalemia on muscle cell pH is unkonwn. Results in other tissues are also unclear. Whether the increase in bicarbonate excretion, reduction in renal ammonia production, and the metabolic acidosis seen in some hyperkalemic states, which is resolved by lowering the potassium level, are attributable to changes in renal cell pH remains hypothetical.

VI. RELATIONSHIP BETWEEN ABNORMAL POTASSIUM LEVELS, CELL pH, AND METABOLISM

A major unanswered question is whether potassium-induced changes in cell pH directly affect metabolism or whether the metabolic changes seen in potassium deficiency and hyperkalemia are unrelated to changes in cellular acidity. As mentioned earlier, urinary ammonia excretion is elevated and citrate excretion reduced in potassium depletion. These changes have been attributed to a reduction in renal cell pH, but direct evidence is lacking. Renal metabolism is altered by acute changes in cell pH, and an acute increase in proximal tubular cell pH *in vitro* decreases both ammoniagenesis and glucose production (Delaney and Adler, 1984). This does not mean, however, that renal metabolic changes seen *in vivo* are due to changes in cell pH. Recently, in our laboratory, we incubated isolated proximal renal tubular cells obtained from potassium-depleted and po-

tassium-repleted rats, determined cell pH by DMO distribution, and measured ammonia and glucose production. At an external pH of 7.50, potassium depletion increased ammonia production by 30% ($P < 0.001$) and glucose production by 35% ($P < 0.001$). Yet, cell pH and the transcellular pH gradient were identical in the two groups. These data may be interpreted in a few ways. It is possible, but probably unlikely, that cell pH plays no role in the renal metabolic changes of potassium deficiency. An alternative explanation is that proximal renal cell pH is reduced *in vivo* in potassium depletion when extracellular potassium concentration is low and proximal tubular cells are hyperpolarized (Cemerikic *et al.*, 1982). In our *in vitro* experiments, however, extracellular potassium was not reduced. Moreover, as shown by Fraley *et al.* (1985), phosphate-dependent glutaminase activity in rat kidney is increased by potassium deficiency. Therefore, potassium deficiency may induce enzymatic changes mediated by altered cell pH. These enzyme alterations might persist *in vitro*, although external bathing media conditions return cell pH to normal. The only direct evidence relating altered tissue metabolism to potassium-induced cell pH changes was obtained *in vitro* by Adler *et al.* (1974c). They measured cell pH and citrate content in intact rat diaphragm muscle acutely potassium depleted *in vitro* and showed that changes in muscle citrate content were best correlated with cell pH and not muscle potassium content or extracellular pH. However, their data also can be explained in other ways. Alterations in local cell potassium concentrations, changes in the transcellular potassium or hydrogen ion ratios, alterations in enzyme induction, or changes in hydrogen ion transport all fit the data. At this time, therefore, it is only possible to conclude that intracellular pH changes induced by alterations in potassium metabolism probably affect cellular metabolism, but the precise mechanisms are unknown. Potassium–hydrogen ion exchange can occur in mitochondria (Garlid, 1980), so changes in cell pH gradients may be altered in ways present methods are yet unable to detect. Further experimentation is clearly necessary to define precisely the relationships between potassium, cell pH, and metabolism.

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Chapter 20

Electrophysiology of Active Potassium Transport across the Mammalian Colon

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I. INTRODUCTION

The large intestine is the site of potassium secretion *in vivo* in a number of mammalian species (Clauss, 1984; Foster *et al.*, 1984; Hawker *et al.*, 1978). Several laboratories have attempted to elucidate the mechanism of potassium secretion by studying the isolated colon *in vitro* under short-circuit conditions (i.e., in the absence of electrical or chemical gradients for K^+ across the epithelium). The decending colon of the rabbit has proved to be a particularly useful model for such studies, as this epithelium is amenable to many different techniques including several electrophysiological methods (cf. Wills, 1984b). In

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addition, this epithelium appears to have several transport properties in common with the human descending colon (Wills *et al.*, 1984).

Initial studies of potassium fluxes across the rabbit descending colon produced conflicting results. On one hand, Yorio and Bentley (1977) and Moreto *et al.* (1981) reported active secretion of this ion. In contrast, Frizzell, Schultz, and coworkers (Frizzell *et al.*, 1976; Frizzell and Schultz, 1978; Schultz, 1981; Fromm and Schultz, 1981) found no net transport of this ion. This investigators concluded that potassium transport was entirely due to a passive mechanism. Because of these discrepant findings, the mechanism of potassium transport and its route of movement across the epithelium were the subject of considerable debate.

Two Pathways for Potassium Transport

A fundamental problem in the study of ion movements across any epithelium is to determine the route of ion permeation. Specifically, does a particular ion cross the epithelium between the cells, i.e., through a paracellular route, or does the ion pass sequentially through the apical and basolateral cell membranes (i.e., by a transcellular route)? From radioisotopic flux determinations and microelectrode studies, Frizzell *et al.* (1976) and Schultz *et al.* (1977) concluded that the apical membrane was impermeable to potassium. Consequently, they proposed that potassium transport was restricted to a potassium-selective paracellular route. In a later study, Fromm and Schultz (1981) proposed that the potassium movement through the junctions occurred by single-file diffusion. The selectivity of this pathway was thought to be regulated such that it is decreased at high luminal potassium concentrations. The latter argument attempted to explain the microelectrode and bi-ionic potential measurements of Wills *et al.* (1979b). These investigators found no evidence for a potassium selectivity in the paracellular pathway.

In a reinvestigation of potassium flux measurements across the colon (Wills and Biagi, 1980, 1982), we found evidence for two active transport systems for potassium across the rabbit descending colon: a net secretory system and a net absorptive system. In agreement with previous studies (Yorio and Bentley, 1977; Moreto *et al.*, 1981), under normal conditions net secretion of potassium was usually obtained (mean $J_K^{net} \sim 0.4$ μEq/cm^2 hr). These investigators reasoned that transcellular potassium secretion might be mediated by the Na^+,K^+-ATPase in the basolateral membrane. Indeed, inhibition of the pump with 10^{-4} *M* serosal ouabain decreased serosal to mucosal movement of potassium.

An unexpected finding after ouabain treatment was a reversal in the direction of net K^+ transport, i.e., net absorption was observed ($J_K^{net} \sim 0.1$ μEq/cm^2 hr). Wills and Biagi (1982) proposed that the direction of net K^+ transport across the colon is determined by the balance between these two oppositely directed active K^+ transport systems. The existence of two oppositely directed K^+ transport

systems could explain previous discrepancies in the literature. Similar results were reported by McCabe *et al.* (1982).

Wills and Biagi (1982) also observed that after ouabain inhibition of the Na–K pump, the permeability of the paracellular pathway was comparable to that for chloride and sodium, measured previously by Frizzell *et al.* (1976). Therefore the paracellular pathway appeared to be nonselective for these ions (i.e., the selectivity of this route was consistent with free diffusional mobility for these ions). The lack of apparent potassium selectivity in the paracellular route confirmed the previous electrophysiological measurements of Wills *et al.* (1979b). Recently, McCabe *et al.* (1984) extensively investigated this issue using radioisotopic techniques and also reported no evidence for potassium selectivity in the paracellular pathway even at high mucosal potassium concentrations. Therefore, it now appears that previous analyses of paracellular potassium transport using this technique were confounded by transcellular movements of potassium in parallel to the paracellular fluxes.

The importance of these findings is that the existence of transcellular mechanisms for potassium transport makes it likely that cellular factors directly regulate net potassium absorption or secretion. More recently, Smith and McCabe (1984a), McCabe and Smith (1985), and Halm *et al.* (1983) have been able to augment net potassium secretion by use of β-adrenergic agents and cAMP. Thus, it appears that potassium transport may be modulated by one or more hormonal systems as well as by other possible factors such as cell metabolism, intracellular pH, and secondary messengers.

II. SIMPLE MODELS FOR ACTIVE K^+ TRANSPORT

Figure 1A and B shows two basic models that can account for net K^+ transport. In Fig. 1A, absorption is shown as an active uptake across the apical membrane, with potassium moving against its electrochemical gradient via an unidentified mechanism. Passive exit of potassium then occurs through a potassium conductance in the basolateral membrane.

Secretion is depicted in Fig. 1B. In terms of this model, potassium is actively taken up (again, against its net electrochemical gradient) from the serosal side of the epithelium via the Na^+,K^+-ATPase in the basolateral membrane. It then passively exits across the apical membrane via a conductive mechanism. The models for absorption and secretion are shown separately. However, recent evidence (Wills, 1985) suggests that both systems could exist in the same cell, as shown in Fig. 1C.

The requirements for the two models are relatively simple. First, for active absorption, there must be an uptake mechanism which uses metabolic energy to drive potassium across the apical membrane against its net electrochemical gra-

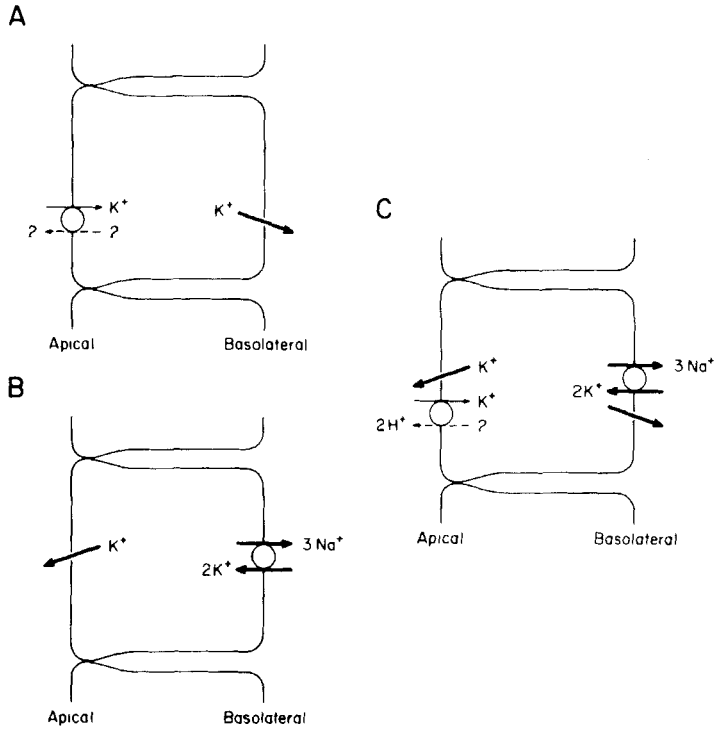

FIG. 1. Simple models for active K^+ transport. (A) Key steps in active K^+ absorption: K^+ movement across the apical membrane against its net electrochemical gradient by an unidentified electroneutral mechanism and passive exit across the basolateral membrane via a conductive mechanism. (B) Key steps in active K^+ secretion: Potassium is actively transported into the cell via an electrogenic Na–K pump (Wills *et al.*, 1979b) and passively diffuses into the lumen across the apical membrane. (C) Hypothetical model showing both transport systems in the same cell.

dient. Second, there must be a sufficient net driving force and conductance to support passive exit of this ion across the basolateral membrane.

Similarly, for active secretion, there must be a significant net electrochemical driving force to support potassium exit across the apical membrane. In addition, the apical membrane must possess a potassium conductance with a magnitude large enough to support the measured potassium fluxes. Another feature implied by the model in Fig. 1B concerns the relationship of K^+ secretion to net Na^+ absorption. Because potassium movements are not limited to a paracellular route, K^+ secretion should be independent of net Na^+ absorption as long as the electrochemical gradient for K^+ across the apical membrane is constant. To investigate this issue, we asked the following questions: (1) How large is the net electrochemical driving force for potassium across the apical membrane under

various conditions? (2) Does the membrane have a potassium conductance and, if so, how large is the conductance?

To answer these questions, we used conventional and ion-sensitive microelectrodes and impedance analysis. Both of these methods have been described in detail in previous reviews (Lewis and Wills, 1981; Wills, 1984b), so the present discussion will be limited to a summary of results from each method. In addition, we note that current fluctuation analysis studies of apical membrane Na^+ channels and apical and basolateral membrane potassium channels in the rabbit descending colon were the subject of a previous review in this series (Wills, 1984b) and will not be discussed in detail here.

III. EVIDENCE FOR THE MODELS

A. The Net Electrochemical Driving Force for Potassium

Figure 2 illustrates the results of intracellular potassium activity measurements (a_iK) performed using K^+-sensitive intracellular microelectrodes. Under short-circuit conditions, Wills and Biagi (1982) found that a_iK averaged 73 ±6 m*M*, with a net electrochemical driving force ($\Delta\mu_K/F$) of 17 ± 3 mV. In preliminary experiments, addition of 10^{-4}*M* amiloride to the mucosal bathing solution hyperpolarized the intracellular potential (V_{Isc}) under these conditions (ΔV_{Isc} = 3 ± 1 mV; n = 3), but did not significantly alter a_iK. Therefore, there was little decrease in $\Delta\mu_K/F$ (−5 ± 2 mV) after amiloride inhibition of Na^+ transport.

Measurements of a_iK in surface cells (Wills, 1985) indicate that the major source of intracellular potassium activity is from the basolateral membrane Na–K pump, since a_iK is depleted by approximately 80% after ouabain addition to the serosal bath. More recently, we assessed whether potassium might also enter across the apical membrane. In brief, we first depleted the cells of K^+ by inhibiting the pump with serosal ouabain (10^{-4} *M*) and bathing the epithelium with K^+-free Ringer's. As shown in Fig. 2, under these conditions, a_iK fell to 5 ± 2 m*M*. Potassium-selective microelectrodes have a measurable selectivity for sodium and other intracellular ions. Therefore, in addition to the remaining intracellular potassium ions, this value presumably reflects intracellular levels of competing ions, particularly Na^+, which is elevated in the presence of ouabain (Wills, 1984a).

We next restored potassium (7 m*M*) to the mucosal bath and a_iK increased to 17 ± 2 m*M*, a value higher than predicted by passive distribution. As indicated in Fig. 2, replacement of the mucosal bath with KCl Ringer's solution (a_0K = 111 m*M*) led to restoration of the basolateral membrane potential and increase in a_iK to 80 ± 13 m*M*. These findings are consistent with active luminal uptake of K^+.

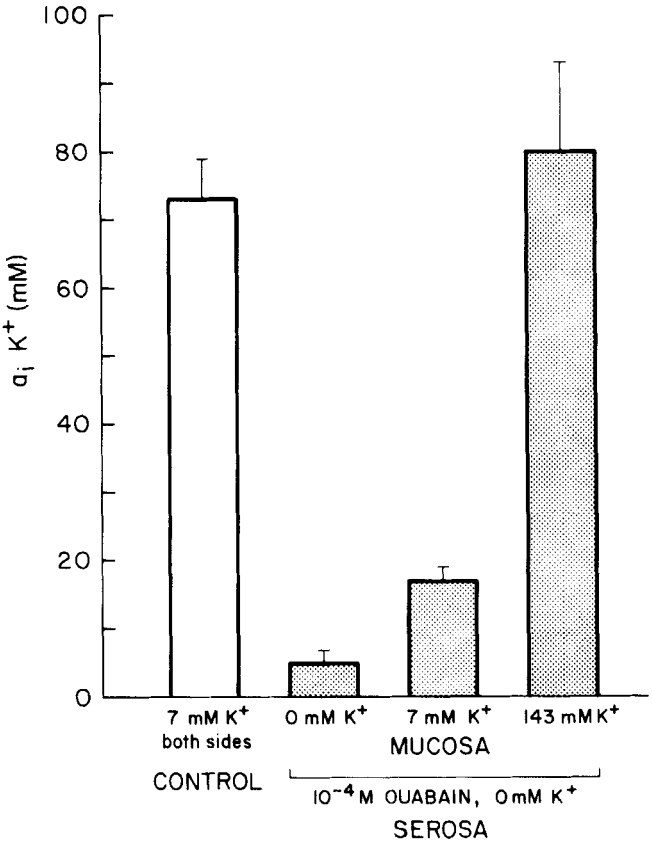

FIG. 2. Mean intracellular potassium activity (a_iK) measured with liquid ion-exchanger microelectrodes. [For description of methods, see Wills (1985).]

B. Features of the Absorptive System

The basolateral membranes of the rabbit descending colon are known to possess a large potassium conductance. This is true for both amiloride-sensitive cells (Wills *et al.*, 1979a; Thompson *et al.*, 1982b) and for the entire epithelium (Wills *et al.*, 1979a,b). Information concerning the apical membrane K^+ uptake mechanism is limited, but K^+ uptake is known to occur against the net electrochemical gradient for K^+ (Wills and Biagi, 1982). Net K^+ absorption is inhibited by metabolic inhibitors such as dinitrophenol (McCabe *et al.*, 1982) or by cooling the epithelium (Wills and Biagi, 1982). However, it persists in the absence of chloride (see Table I) and is not inhibited by loop diuretics (Wills, unpublished observations) or mucosal ouabain (Wills *et al.*, 1982; McCabe *et al.*, 1982). Therefore K^+ absorption does not appear to involve an apical membrane Na^+,K^+-ATPase or a cotransport mechanism coupled to chloride. Gustin

TABLE I
^{42}K FLUXES IN Cl^--FREE SOLUTIONS

	J_{ms} (μEq/cm^2 hr)	J_{sm} (μEq/cm^2 hr)	J_{net} (μEq/cm^2 hr)
Sodium gluconate + ouabain (10^{-4} *M* serosa), $n = 4$	0.307 ± .056	0.102 ± .019	0.205 ± .070

and Goodman (1981) reported evidence for a H^+,K^+-ATPase in isolated brush-border membranes from the rabbit descending colon. So far, there has been no direct proof of H–K exchange in the intact tissue. Some preliminary indirect evidence for a H^+,K^+-ATPase in colonic epithelia comes from the work of Suzuki (1983) who demonstrated H^+ secretion by the guinea pig colon. Although this secretion required potassium, it was also inhibited by ouabain. Therefore this system may differ from that found in the rabbit.

In the remainder of this chapter, we will focus on the K^+ secretory system, since this is the mode of net transport most typically observed in this epithelium and for this reason has been studied more extensively.

C. The Secretory System

1. Apical Membrane Amiloride-Insensitive Conductance

The apical membranes of the rabbit descending colon epithelial cells were initially assumed to be exclusively conductive for sodium (Schultz *et al.*, 1977). Current fluctuation analysis studies have established that this conductance is due to amiloride-blockable Na^+ channels (Zeiske *et al.*, 1982). Microelectrode studies by Wills *et al.* (1979b) and Thompson *et al.* (1982a,b) demonstrated a significant amiloride-insensitive conductance in the apical membrane of these cells in parallel to the Na^+ channels.

Recently, an alternate explanation of the amiloride-insensitive conductance was offered by Nagel *et al.* (1983). They suggested that distributed resistance effects (Clausen *et al.*, 1979; Boulpaep and Sackin, 1980) might significantly influence membrane resistance ratio measurements and thereby artifactually overestimate apical membrane conductance. Therefore, we decided to examine the extent of distributed resistance effects using impedance analysis and the distributed resistance model (Clausen *et al.*, 1979). In this model, the basolateral membrane resistance is distributed along the lateral intracellular space (see equivalent circuit, Fig. 3). We examined three conditions: (1) "control" conditions (NaCl Ringer's solution, both sides); (2) 10^{-4} *M* amiloride; and (3) Na and Cl replacement (potassium gluconate or K_2SO_4 bicarbonate Ringer's solution; see Wills, 1985). The results are given in Table II.

As reported previously (Clausen and Wills, 1981; Wills, 1984b), under most

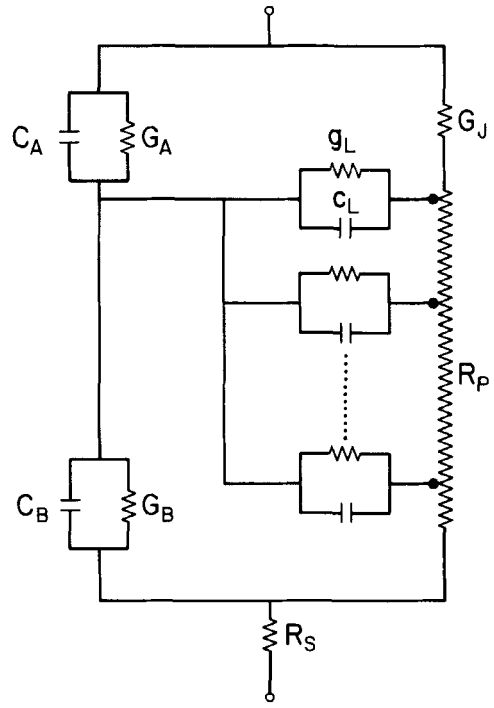

FIG. 3. The distributed impedance model of Clausen *et al.* (1979). The subscripts A, B, and L refer to the apical, basal, and lateral membranes, respectively. G and C are conductances and capacitors, respectively. R_p is the resistance of the lateral intracellular space and R_S is the series resistance.

conditions the data were reasonably fitted by a "lumped" model; i.e., no significant distributed resistance effects were noted. This is reflected in Table II for the amiloride and control conditions by the small values for the lateral intercellular space resistance (R_p) compared to the basolateral membrane resistance ($1/G_b$). The lack of a distributed resistance is also reflected by membrane resistance ratio measurements (R_a/R_{bl}). R_a/R_{bl} values estimated from impedance measurements were in good agreement with those reported previously from microelectrode studies (Wills *et al.*, 1979b). Consequently, distributed resistance effects do not account for the relatively low R_a/R_{bl} values in the presence of amiloride reported previously by Thompson *et al.* (1982a) and Wills *et al.* (1979b).

Wills *et al.* (1979a) and Thompson *et al.* (1982a,b) used equivalent circuit analysis and microelectrode measurements to estimate the magnitude of the resistance of the amiloride-insensitive pathway as ~1.6–1.7 kΩ·cm^2. Table II shows apical membrane conductances normalized to membrane area (capacitance in microfarads where 1 μF ~ 1 cm^2 of actual membrane area) for the same

TABLE II

MEMBRANE CONDUCTANCES AND RESISTANCE RATIOS (R_a/R_{bl}) ESTIMATED FROM IMPEDANCE ANALYSIS USING LIS DISTRIBUTED MODEL

Condition	G_a (mS/μF)	G_{bl} (mS/μF)	R_p ($\Omega \cdot cm^2$)	R_a/R_{bl}[a] (impedance)	R_a/R_{bl}[b] (microelectrode)	R_a/R_{bl}[c] ("corrected" microelectrode)
Control (NaCl Ringer's) $n = 10$	0.19 ± 0.03	1.05 ± 0.07	15 ± 1	4 ± 1	4 ± 1	4.6
Amiloride (10^{-4} *M* mucosa), $n = 4$	0.09 ± 0.02[d]	0.86 ± 0.14[d]	13 ± 1	8 ± 1[d]	10 ± 3	12.2
K^+ Ringer's (143 m*M* K^+ gluconate or K_2SO_4), $n = 8$	0.09 ± 0.01[d]	2.73 ± 0.13[d]	60 ± 10[d]	33 ± 14[d]	8 ± 1	12.7

[a] Calculated as $R_a/R_{bl} = G_{bl}/G_a$ from impedance values of G_a (mS/cm²) and G_{bl} (mS/cm²).

[b] From Wills *et al.* (1979a).

[c] Corrected using Eq. (1) in text and mean values of R_a/R_{bl} (microelectrode).

[d] $P < 0.05$ compared to control.

conditions. Note that a significant conductance was present in the apical membrane in the presence of amiloride, in agreement with an amiloride-insensitive apical membrane conductance. In terms of resistance ($R = 1/G$), this conductance was ~11 k$\Omega \cdot \mu$F, or normalized to nominal chamber area, about 529 $\Omega \cdot cm^2$. This resistance is lower than that estimated by previous studies.

The source of this apparent discrepancy between the two estimates can be understood if we consider that microelectrode methods measure membrane properties for only the impaled cell type. In DC equivalent circuit analysis of epithelia that have more than one cell type (such as the colon), the conductances of other cell types are lumped into the paracellular conductance (for discussion, see Wills *et al.*, 1979b). In contrast, impedance methods measure the net membrane properties of the entire epithelium. Consequently, the apical membrane conductance of parallel cell types (e.g., crypt cells) are included in the estimate of apical membrane conductance as measured using impedance analysis, but is not included in microelectrode measurements of this conductance. The lower resistance estimate for the impedance data suggests that such cells also possess an amiloride-insensitive apical membrane conductance. This finding is consistent with the results of Welsh *et al.* (1982), who found evidence for an apical membrane chloride conductance in crypt cells.

In summary of the results for control and amiloride-treated tissues, two amiloride-insensitive pathways appear to be present: (1) a pathway in the same cell membrane as the (amiloride-sensitive) Na^+ channels, and (2) another parallel pathway, perhaps in crypt cells. Distributed resistance effects were minimal under these conditions.

When do distributed resistances significantly affect microelectrode measurements of membrane resistance ratios in the colon? Inspection of Table II reveals that the lateral intercellular space resistance (R_p) was increased by approximately fourfold when sodium and chloride in the mucosal bathing solutions were replaced by potassium and impermeant anions. Under these conditions, there was a large discrepancy between the impedance determined values and that measured previously using microelectrodes in surface epithelial cells. Again, it is useful to recall that impedance measurements reflect more than one cell type. In contrast, microelectrode measurements (carried out in surface cells) measure only one cell type, in this case, the so-called absorptive cells or amiloride-sensitive cells. Nonetheless, we can use estimates of the lateral intercellular space resistance determined by impedance analysis to evaluate the extent of distributed resistance effects on the measured microelectrode resistance ratios. As a first approximation, we estimated a "corrected" microelectrode measurement of R_a/R_{bl} using the following equation:

$$(R_a/R_{bl})_{corrected} = 1/(R_a/R_{bl})_{measured} - 1/3\ (R_p/R_a) \quad (1)$$

where $(R_a/R_{bl})_{measured}$ is the resistance ratio from microelectrode experiments

(Wills *et al.*, 1979b) and R_a and R_p are apical membrane resistance and lateral intercellular space resistance determined from impedance measurements. Calculated in such a manner, $(R_a/R_{bl})_{corrected}$ was ~13, given an initial $(R_a/R_{bl})_{measured}$ value of 8 for the K^+ Ringer's condition. Assuming that the basolateral membrane resistance is the same as determined by microelectrode studies (Wills *et al.*, 1979b; Thompson *et al.*, 1982a,b), this finding is consistent with a minimum potassium conductance of 0.47 mS/cm^2 in the apical membranes of surface cells for high mucosal potassium conditions ($[K^+]$ = 143 m*M*). We note that similar corrections of R_a/R_{bl} for the control and amiloride condition also resulted in small increases. Therefore, these calculations suggest that the maximum value for the apical membrane amiloride-insensitive resistance is ~2 kΩ·cm^2.

Evidence for an apical membrane potassium conductance in surface cells also comes from ion-sensitive microelectrode studies. Wills (1985) measured the chemical driving forces for potassium and chloride across the apical membrane and compared these to previous estimates of the electromotive force (emf) for the amiloride-insensitive pathway (Wills *et al.*, 1979b; Thompson *et al.*, 1982a,b). Previous estimates for this emf were −57 and −51 mV, respectively. The Na^+ gradient is in the wrong direction to account for such an emf. The chloride chemical driving force averaged −38 ± 2 mV, a value too low to support the emf. In contrast, the potassium gradient (−69 mV) was more than adequate to account for the amiloride-insensitive emf.

Is the amiloride-insensitive conductance large enough to support the net secretion of potassium measured in radioisotopic flux studies? Wills and Biagi (1982) estimated that the potassium conductance of surface cells was essentially large enough to account for the secretion. As indicated in Table II, the present impedance measurements also indicate an apical membrane potassium conductance which is adequate to support net K^+ secretion. Moreover, it appears that an additional parallel conductance may be present in amiloride-insensitive cells. Therefore, it is conceivable that more than one K^+ secretory system may be present in the colon.

Another important aspect of apical membrane properties was recently reported by Wills *et al.* (1982) who used current fluctuation analysis to identify apical membrane potassium channels in this epithelium. These channels were partially blocked by Cs^+ and tetraethylammonium ions, but not barium. It is likely that at least part of the secretion may be mediated by this channel; however, further evaluation of this question will require the identification of a more effective channel blocking agent.

2. Basolateral Membrane Properties

Microelectrode studies of the rabbit descending colon demonstrated a large basolateral membrane potassium conductance (Thompson *et al.*, 1982b). This

conductance was previously studied in so-called single-membrane preparations of this epithelium using the polyene antibiotic nystatin (Wills *et al.*, 1979a,b). As shown in Fig. 4, this preparation involves bathing the mucosal side of the epithelium with a potassium Ringer's solution designed to match intracellular potassium activity and which contains no permeable anions. When a maximally effective dose of nystatin is added to the membrane, the apical membrane resistance can be selectively abolished without significantly altering either the paracellular or basolateral membrane resistances (if appropriate anions are used; see Chapter 3, this volume). Under these conditions, the preparation electrically resembles a basolateral membrane resistance in parallel to the paracellular shunt resistance.

Wills *et al.* (1979a,b) used bi-ionic potential measurements and current–voltage relationships to assess basolateral membrane-selective permeability for sodium, chloride, and potassium. The ratios of sodium to potassium permeability (P_{Na}/P_K) and chloride to potassium permeability (P_{Cl}/P_K) estimated from the two methods were both approximately equal to 0.05. Potassium permeability (P_K) was $\sim 100 \times 10^{-6}$ cm/sec. These selectivities are similar to those reported by Thompson *et al.* (1982b) from current–voltage relationships and equivalent circuit analysis.

The existence of a finite basolateral membrane conductance for sodium suggests that at least part of the intracellular sodium pool arises from the serosal side of the epithelium. While this arrangement may seem inefficient, it may explain the ability of surface colonic cells to maintain intracellular potassium levels above electrochemical equilibrium even when entry of sodium across the apical membrane is blocked by amiloride. Similar results of a serosal source of a_iNa

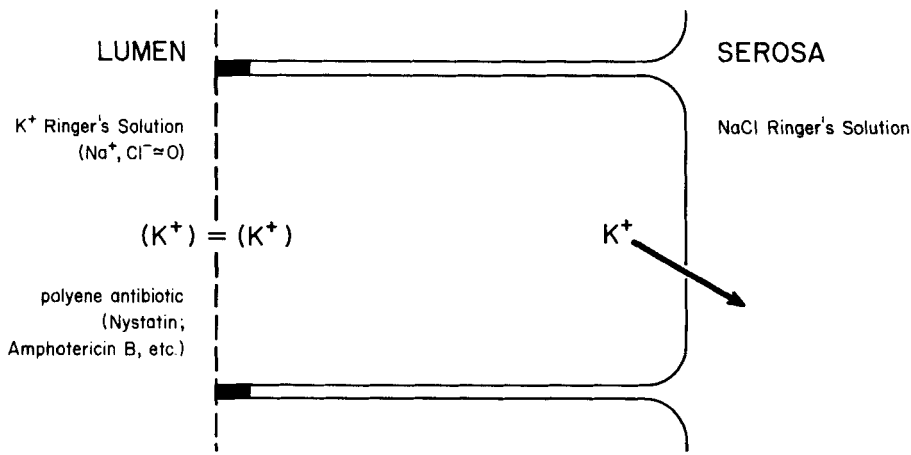

FIG. 4. Diagram of experimental conditions for the "single-membrane" preparation of the colon. For details, see text.

have been obtained for the rabbit urinary bladder by Lewis and Wills (1983) using intracellular Na^+-specific microelectrodes. Additional electroneutral exchange of Na^+ across the basolateral membrane cannot be excluded.

Recent studies (Wills, 1985) have shown that the basolateral membrane conductance can be blocked by serosal Ba^{2+} addition. Current fluctuation analysis experiments in single-membrane preparations (Van Driessche *et al.*, 1982) have revealed a spontaneously fluctuating current signal with characteristics similar to potassium channel noise from the frog skin (Van Driessche and Zeiske, 1980). This signal could also be reversibly blocked by serosal Ba^{2+} (cf. Wills, 1984b).

IV. SUMMARY

Figure 5 summarizes the above findings into a revised model of colonic K^+ transport. Key features of the model include parallel active K^+ uptake mecha-

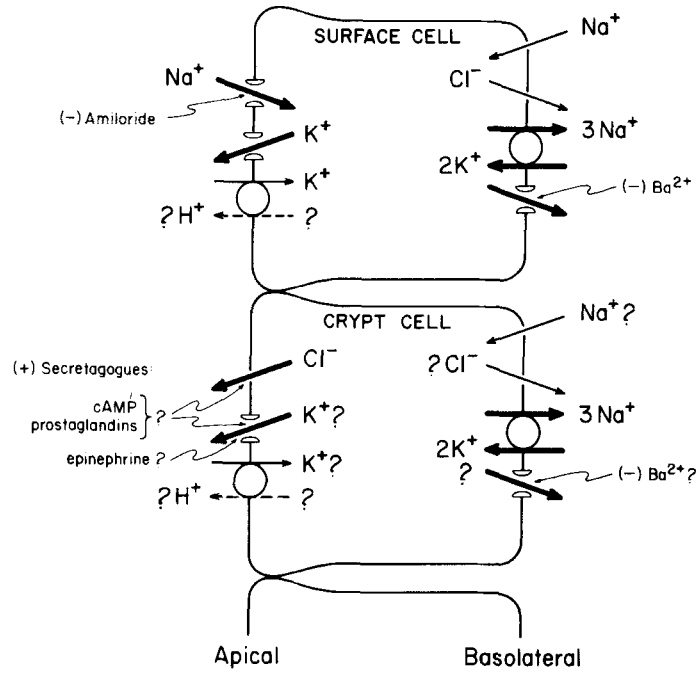

FIG. 5. Revised model for potassium transport across the rabbit colon for surface and crypt epithelial cells. Both cells are shown with "pump-leak" transport mechanisms for potassium. These may be the same or different mechanisms in the two cell types. Secretagogue effects are tentatively assigned to crypt cells. Basolateral membrane properties for crypt cells have not been evaluated.

nisms and conductances in the basolateral and apical membranes. Consequently, the direction of net potassium transport will depend on the combined rates of potassium exit and uptake across both membranes. The K^+ conductances are represented as channel mechanisms. Further work is needed to evaluate the factors which regulate these channels and to identify the mechanism of active K^+ uptake across the apical membrane. The site of action of secretagogues, such as prostaglandins E_1 and E_2, epinephrine, and cAMP, needs to be resolved, as well as the possible role of crypt cells in potassium transport.

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Chapter 21

Potassium Adaptation in Mammalian Colon

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I. PHENOMENA OF POTASSIUM ADAPTATION

An adaptive response to avoid potassium intoxication during periods of heavy potassium intake was first noted by Thatcher and Radike (1947). They reported that rats chronically treated with large doses of potassium were resistant to an acute oral dose of potassium that was lethal to control animals. Subsequently, Berliner *et al.* (1950) demonstrated that the rate of urinary excretion of potassium was increased above control during the acute infusion of KCl to dogs fed a potassium-enriched diet for 2 weeks. Similar observations were made in the rat chronically fed a high potassium diet (Alexander and Levinsky, 1968) and in animals with renal insufficiency fed a normal intake of potassium (Schon *et al.*, 1974). Since high rates of urinary potassium excretion were shown to result from potassium secretion in distal portions of the nephron (Malnic *et al.*, 1964), chronic potassium loading presumably increased the maximum capacity for tubular secretion of potassium in those nephron segments. Recent studies have demonstrated that potassium secretion is increased above control in potassium-loaded animals in the initial collecting tubule (Wright *et al.*, 1971), the cortical collecting tubule (Fine *et al.*, 1979) and the medullary collecting duct (Schon *et al.*, 1981) of the mammal. This alteration in tubular secretion has been termed "potassium adaptation" (Hayslett and Binder, 1982).

Since the mammalian colon is characterized by net potassium secretion *in vivo* (Edmonds and Marriott, 1968; Phillips, 1969), Fisher *et al.* (1976) examined the response of the rat colon to chronic potassium loading to determine whether the

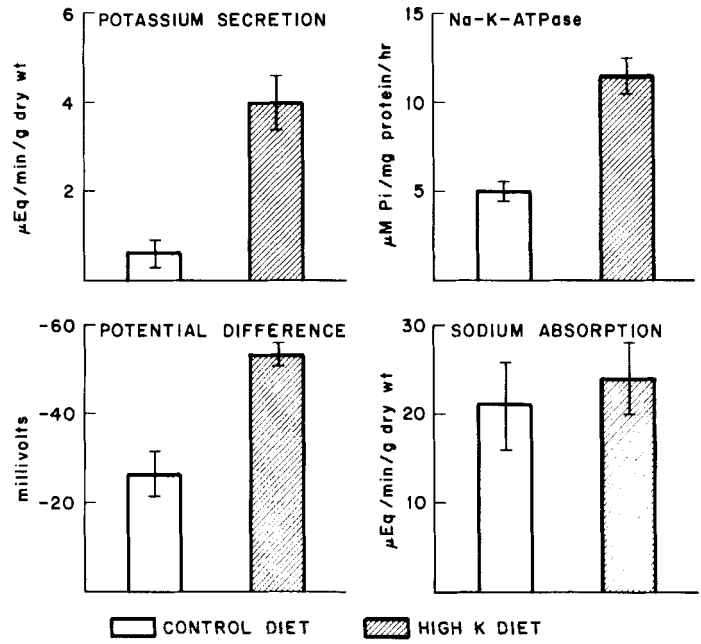

FIG. 1. Comparison of net cation transport, transmural potential difference *in vivo*, and Na^+,K^+-ATPase activity in rat colon, in controls, and in potassium-loaded animals. Values are mean ± SEM. [From Fisher *et al.* (1976).]

phenomenon of potassium adaptation was demonstrable in another epithelium capable of potassium secretion. After 7 days of a high potassium diet, potassium secretion was markedly increased *in vivo* in rat colon from 0.8 to 3.9 μEq/hr per gram dry weight, and transmural potential difference (PD) rose twofold from a mean value of 26 to 54 mV (lumen negative), as shown in Fig. 1. Subsequent studies showed that the *in vivo* increase in potassium secretion caused by a high potassium diet occurred in both proximal and distal portions of colon (Kashgarian *et al.*, 1980). It was of interest that under *in vivo* conditions, there was no significant change in net movement of Na, Cl, or HCO_3 in potassium-adapted animals.

II. STIMULATION OF ACTIVE POTASSIUM SECRETION IN POTASSIUM ADAPTATION

At the time these studies were performed, the mechanism of potassium secretion in mammalian colon was controversial. Previous studies under *in vitro* conditions in several species in the absence of an electrical gradient demonstrated

either no evidence of active potassium transport (Frizzell *et al.*, 1976; Frizzell and Schultz, 1978), or small, but statistically significant net and presumably active potassium movement (Bentley and Smith, 1975; Yorio and Bentley, 1977). To determine the mode of potassium secretion in control and potassium-adapted rats, Kliger *et al.* (1981) examined the distribution of potassium across the colonic mucosa when net water and electrolyte movement approached zero under *in vivo* conditions. The results indicated that in control animals active secretion of potassium was present in proximal colon against an electrochemical gradient of ~31.9 ± 1.3 mV, whereas in distal colon, absorption occurred against a gradient of −16.7 ± 2.2 mV. In potassium-adapted animals active absorption in distal colon was transformed to active secretion, and the observed luminal concentrations of potassium were found to be approximately four- to sixfold higher than those predicted from the electrochemical driving force at equilibrium in both segments of colon. The calculated active transport potential in adapted animals was +46.7 ± 0.9 and +54.1 ± 2.7 mV in proximal and distal segments, respectively.

The mechanism of potassium transport in colon from control and potassium-loaded animals was also studied under *in vitro* conditions in which the electrical gradient was eliminated by short-circuiting. In agreement with the *in vivo* observations, there was net potassium secretion in control proximal colon (−0.23 ± 0.05 μEq/hr·cm^2), which increased to −0.79 ± 0.17 μEq/hr·cm^2 ($P < 0.001$) in the experimental group (9). In distal colon, potassium absorption (+0.43 ± 0.10 μEq/hr·cm^2) was reversed to net secretion (−0.76 ± 0.08 μEq/hr·cm^2, $P < 0.001$) in the group administered the potassium-enriched diet (Foster *et al.*, 1984), as shown in Fig. 2. Chronic potassium loading therefore stimulated active potassium secretion in proximal colon and induced active potassium secretion in distal colon. Regarding the possible dependency of potassium secretion on the reciprocal absorption of sodium, it was found that net potassium secretion was unaltered after net sodium absorption was abolished by addition of amiloride to the mucosal bath solution (Foster *et al.*, 1984). Moreover, under this condition the short-circuit current was equal to net potassium secretion, indicating that potassium secretion was electrogenic.

Potassium secretion in the mammalian colon is modulated by a potential-dependent mechanism via the paracellular shunt pathway and by a potential-independent mechanism since net secretion was demonstrable in the absence of an electrical gradient. Active potassium secretion indicates a transcellular route for transfer across the epithelium. Independent evidence for an important role of transcellular movement of potassium was advanced by the demonstration that the process of net potassium secretion in rat colon was saturable at extracellular potassium levels of ~10 mEq/liter, suggesting a carrier-mediated mechanism (Hayslett *et al.*, 1982). Further studies were performed to elucidate the cellular mechanism governing potassium movement in controls and the increased rate of secretion in potassium-loaded animals.

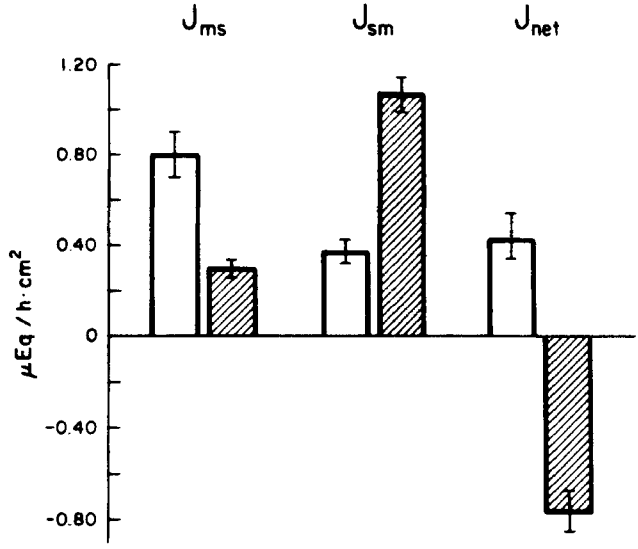

FIG. 2. Demonstration of transformation from active potassium absorption in controls (clear bars) to active potassium secretion in potassium-loaded (hatched bars) animals in distal colon under *in vitro* conditions. Values are mean ± SEM. [From Foster *et al.* (1985).]

III. INCREASE IN Na^+,K^+-ATPase IN POTASSIUM ADAPTATION

It is generally accepted that the composition of cytosol, characterized by a low sodium concentration and high concentration of potassium, is regulated by Na^+,K^+-ATPase situated in the basolateral cell membrane. This feature indicates that cell uptake of potassium takes place primarily across the cell surface facing the interstitial fluid compartment. In the steady state of metabolizing cells, the constant level of intracellular potassium requires that potassium leaves the cell as rapidly as it enters. The direction and rate of a potassium leak from the cell depends on the electrochemical gradient for passive diffusion and potassium conductances of the apical and basolateral cell membranes, and possibly other carrier-mediated processes involving potassium transport in the two asymmetrical cell surfaces. Translocation of potassium from interstitial to luminal fluid via a transcellular route requires a finite potassium conductance in the apical membrane, and this property was established by demonstration of active transport across the whole epithelium.

Our initial efforts to determine the mechanism of potassium adaptation focused on the potassium pump in basolateral membrane. Silva *et al.* (1973) provided the first evidence suggesting that potassium secretion was mediated by Na^+,K^+-ATPase by demonstrating a marked increase in Na^+,K^+-ATPase ac-

tivity above control levels in both homogenates of renal tissue and in membrane-rich fractions of kidney tissue from potassium-loaded animals. Analysis of colonic mucosa by Fisher *et al.* (1976) under the same conditions showed that Na^+,K^+-ATPase activity was also significantly increased in colon by potassium loading (Fig. 1). This change was specific for potassium-secreting epithelia, since similar alterations in enzyme activity were not found in brain, liver, diaphragm muscle, or in jejunum. Moreover, in tissues characterized by increased Na^+,K^+-ATPase activity, there was no change in Mg-ATPase or in 5′-nucleotidase used as another marker of the basolateral cell membrane. Since it was shown that the urinary fractional excretion of potassium increased in renal insufficiency to preserve potassium balance in animals on a normal potassium intake (Schon *et al.*, 1974), it was of interest that the activity of Na^+,K^+-ATPase increased in renal cortex and medulla (Schon *et al.*, 1974) and in colonic mucosa (Bastl *et al.*, 1977) of rats with moderate renal insufficiency induced by surgical ablation of renal tissue. These findings were interpreted as suggesting that cell uptake of potassium was increased in potassium-adapted epithelia due to the greater availability of potassium pumps in basolateral cell membrane.

Further studies were performed to determine whether the change in Na^+,K^+-ATPase activity was caused by a qualitative alteration in the enzyme or an actual rise in the number of pump units, whether the quantitative change arose from an increased area of basolateral cell membrane and/or a change in the density of pump sites per unit of cell membrane. As shown in Fig. 3, specific [^{3}H]ouabain binding increased 38 ± 9% in plasma membrane-rich fractions of rat colon from potassium-loaded animals compared to control and correlated with a similar rise in Na^+,K^+-ATPase activity of 40 ± 10% in the same experimental group (Hayslett *et al.*, 1980). The change in ouabain binding was not due to an increase in affinity for ouabain in experimental tissue, since the K_D of ouabain binding

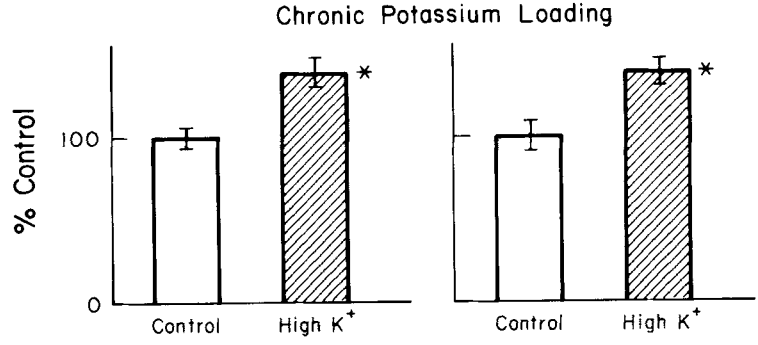

FIG. 3. Effect of chronic potassium loading to increase Na^+,K^+-ATPase activity (left) and specific [^{3}H]ouabain binding (right) in rat colon. * indicates $P < 0.001$. Values are mean ± SEM. [From Hayslett *et al.* (1980).]

(12 μM) was similar in control and experimental tissue, and Scatchard analysis demonstrated a significant increase in binding sites per milligram of membrane protein. Additional studies demonstrated that there was no evidence in experimental tissue of an increase in the K_m of Na^+,K^+-ATPase for ATP (Hayslett *et al.*, 1980) or potassium (Fisher *et al.*, 1976) to account for the observed increase in enzyme activity. To determine whether there was an increase in the density of K pump sites in basolateral cell membrane, Na^+,K^+-ATPase activity was compared with activity of adenylate cyclase, since adenylate cyclase has been shown to localize in the basolateral membrane of rat intestinal tissue (Hayslett *et al.*, 1980). Na^+,K^+-ATPase activity per milligram membrane protein increased significantly, whereas the activity of adenylate cyclase was unaltered. Taken together, these data clearly demonstrate that potassium loading results in an increase in both the number and density of enzyme transport units.

IV. AMPLIFICATION OF THE AREA OF BASOLATERAL CELL MEMBRANE

Morphometric studies were performed by Kashgarian *et al.* (1980) to determine whether the increase in number of pump sites correlated with an amplification of the area of cell membranes. Inspection of photomicrographs of rat colonic epithelia in control animals demonstrated marked differences in cell morphology between proximal and distal colon. Cells in proximal rat colon were larger in height (36.8 $\pm$ 1.1 μm) and in width (5.5 $\pm$ 0.2 μm) compared to distal colon where cell dimensions were 21.0 $\pm$ 0.8 μm in height and 4.0 $\pm$ 0.2 μm in width. A difference between proximal and distal colon was also found when cell membrane density was determined by stereologic techniques. The basolateral membrane density (S_V) was greater in distal colon (1.85 $\pm$ 0.8 μm^2 per μm^3) than the value in proximal colon of (1.48 $\pm$ 0.04, $P < 0.001$), and the surface density of microvilli on luminal membrane (S_S) was also greater in distal (33.2 $\pm$ 1.1 μm^2 per μm^2) than in proximal colon (19.3 $\pm$ 1.5, $P < 0.001$).

Chronic potassium loading resulted in a marked increase in infolding of basolateral cell membrane of mucosal cells in both proximal and distal colon and correlated directly with changes in net potassium secretion and the activity of Na^+,K^+ ATPase. In experimental animals, S_V rose 38% in proximal colon and 37% in distal colon, as shown in Fig. 4. The structural change observed in the group of animals with stimulated potassium secretion was restricted to the basolateral cell membrane, since the villus surface density, S_S, was not greater compared to controls. It was of interest that in these studies, experiments were performed to determine whether the morphological changes associated with increased potassium secretion were common to other conditions besides chronic potassium loading which were characterized by augmented rates of potassium

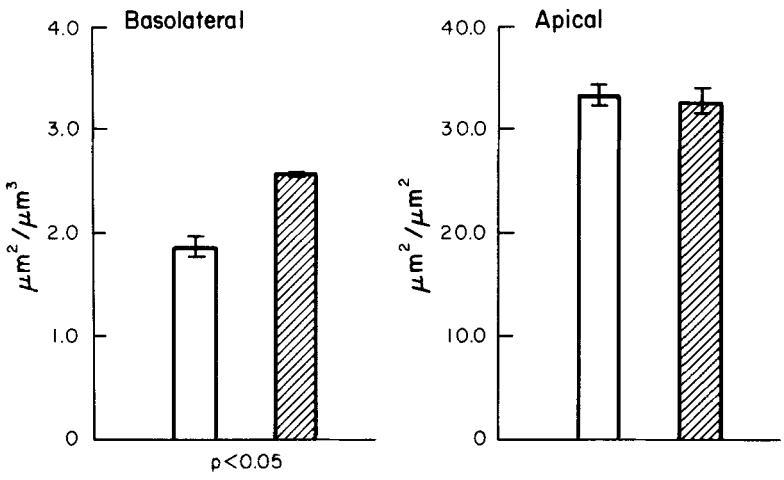

FIG. 4. Effect of chronic potassium loading to increase basolateral membrane surface density (S_V) in rat distal colon, in absence of a change in apical surface density (S_S). Values are mean ± SEM. Clear bar, control; cross-hatched bar, chronic K loading. [From Kashgarian *et al.* (1980).]

secretion. An analysis of the morphological changes in chronic hyperaldosteronism and following treatment with the glucocorticoid hormone dexamethasone demonstrated a direct correlation between an increase in S_V of the basolateral membrane and net potassium secretion. Inspection of electron micrographs also demonstrated that mitochondria were larger and the density of the cristae appeared to be increased in experimental tissue, suggesting that mitochondrial aerobic metabolism might provide for a higher rate of ATP production as a substrate for Na^+,K^+-ATPase and thus serve as a source of energy for the increased potassium secretion in the various adapted states studied.

On the basis of the observation that the area of basolateral cell membrane increased in association with a chronic increase in potassium transport and transport enzyme activity, we suggested that each new pump might require additional phospholipid arranged in an orderly fashion to electrically isolate one ion transport site from another during the process of translocation of electrically charged particles (Kashgarian *et al.*, 1980). Although these studies provided the first demonstration in the mammal that the area of basolateral membrane of epithelia increased in physiological states characterized by augmented rates of cation transport, previous studies in lower vertebrates demonstrated similar changes in the area of the basolateral membrane in renal tubules (Wendelaar *et al.*, 1974) and nasal salt glands (Ernst, 1972) under conditions when sodium secretion was chronically increased. Subsequent to these studies, an increase in basolateral membrane area was demonstrated in the epithelial cells of the initial collecting tubule (Stanton *et al.*, 1981) and outer medullary collecting duct (Rastegar *et al.*,

1980) of potassium-loaded animals. In both of these nephron segments, as noted above, potassium secretion was increased above control after administration of a potassium-enriched diet (Schon *et al.*, 1981; Wright *et al.*, 1971).

V. ELECTROPHYSIOLOGICAL CHANGES IN POTASSIUM ADAPTATION

Since transfer of cellular potassium across the apical membrane is regulated by the elctrochemical driving forces acting on the potassium ion and the conductance for potassium in cell membranes, further experiments were performed in rat colon prepared as a flat sheet in order that cellular impalements could be made (Sandle *et al.*, 1985). Compared to control tissue, potassium loading resulted in a 5-fold increase in transepithelial potential difference (V_T) (15 ± 2 versus 3 ± 1 mV, lumen negative, $P < 0.001$), a 15-fold increase in short-circuit current (I_{sc}) (185 ± 29 versus 12 ± 2 $\mu A \cdot cm^{-2}$, $P < 0.001$), and a 52% decrease in epithelial resistance (R_t) (209 ± 11 versus 101 ± 9 $\cdot cm^2$, $P < 0.001$). The rise in V_T was associated with an increase in V_{BL} from 45 ± 2 to 56 ± 2 mV, $P < 0.001$, in the absence of a change in V_A compared to the control value of 42 ± 2 mV.

Using potassium-sensitive microelectrodes, the activity of potassium in controls was demonstrated to be 86 ± 4 m*M*, as shown in Fig. 5. As the calculated potassium equilibrium potential (E_K) across the basolateral membrane (−74 ± 2 mV) in control tissue was greater than the V_{BL} of 43 ± 2 mV, these data indicate that cell uptake of potassium across the basolateral membrane occurred against a chemical gradient and hence was an active process (Sandle *et al.*, 1985). This result demonstrates that V_{BL} is primarily a potassium diffusion potential. In potassium-loaded animals, cellular potassium activity was 153 ± 12 m*M*, and the E_K of −88 ± 2 mV was again higher than V_{BL} (54 ± 3 mV, $P < 0.001$). Since E_K and V_{BL} increased proportionally above control values, potassium was transported across basolateral membrane against an electrochemical gradient of ~30 mV in both controls and tissue from potassium-loaded animals. These data also show, however, that the electrochemical gradient favoring passive diffusion of potassium across the apical membrane increased in experimental tissue from 32 to 47 mV.

To determine the effect of potassium loading on sodium and potassium conductance of apical membrane, we tested the inhibitory action of the sodium channel blocker amiloride and the potassium channel blocker tetraethylammonium (TEA) on the ratio of R_a/R_{bl} (α) in controls and tissue from experimental animals. The value of α was significantly lower in experimental tissue (2 ± 1) than in controls (14 ± 3, $P < 0.002$), suggesting that potassium loading increased apical membrane conductance. Netiher amiloride nor TEA altered α in

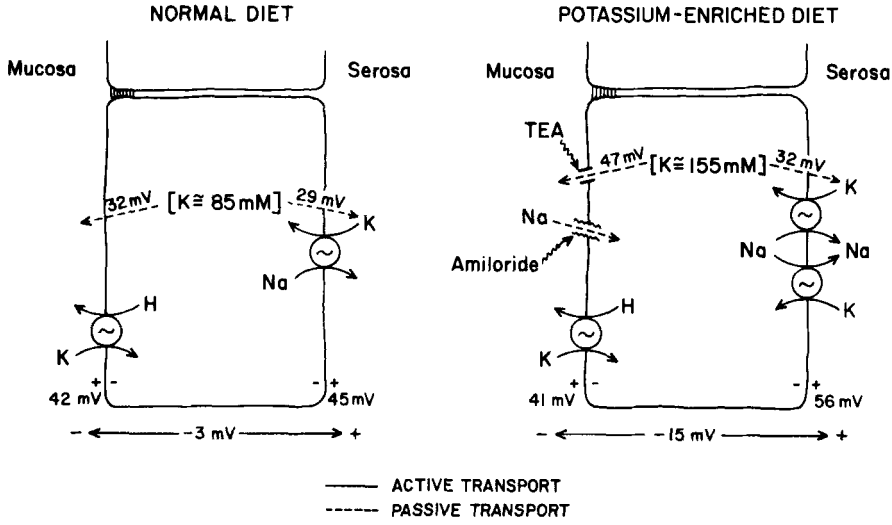

FIG. 5. Proposed model for potassium transport in distal colon in controls and potassium-loaded animals. In these experiments, the activities of potassium and sodium in the bathing solution were 5.7 m*M* and 103 m*M*, respectively. (——), Active transport; (- - -), passive transport. [From Sandle *et al.* (1985).]

control colon. In experimental tissue α increased to 5 ± 1 ($P < 0.05$) following the addition of amiloride, and further to 14 ± 2 ($P < 0.005$) after the addition of TEA to mucosal solution. These data are consistent with the notion that potassium loading resulted in an increase in both sodium and potassium conductance in apical cell membrane.

VI. ROLE OF ALDOSTERONE AND POTASSIUM IN THE INDUCTION OF POTASSIUM ADAPTATION

Although these studies have characterized the epithelial changes involved in potassium adaptation in the colon, the stimulus responsible for these alterations in epithelial transport is unknown. Because chronic potassium loading increases aldosterone production and elevates plasma aldosterone levels (Martin *et al.*, 1983), it was not known whether the adaptive changes produced by potassium loading were due solely to hyperaldosteronism, represented a unique effect of chronic potassium loading, or occurred as a result of the interaction of dietary potassium loading and hyperaldosteronism. To distinguish between the individual effects of aldosterone and chronic potassium loading in the mechanism of

potassium adaptation, studies were performed in distal colon that permitted systematic manipulation of serum aldosterone levels and potassium loading.

Using an experimental model recently described by this laboratory (Martin *et al.*, 1983), adrenalectomized animals were administered corticosterone in an amount that restored basal plasma levels and varying amounts of aldosterone and were fed either the standard or a potassium-enriched diet (Foster *et al.*, 1985). The effect of aldosterone on the potassium-adaptative process was evaluated by measuring unidirectional ^{42}K fluxes under short-circuit conditions across distal colonic mucosa. In the absence of elevated aldosterone levels in adrenalectomized animals fed a normal diet and with basal replacement of glucocorticoids, the rate of net potassium absorption ($+0.43 \pm 0.07$ $\mu Eq/hr \cdot cm^2$) was similar to the rate found in control tissue from adrenal intact animals. Both hyperaldosteronism and administration of a high potassium diet reduced net potassium absorption to zero (-0.03 ± 0.06 $\mu Eq/hr \cdot cm^2$ and -0.14 ± 0.08 $\mu Eq/hr \cdot cm^2$, respectively), but neither maneuver alone caused net potassium secretion. In contrast, the administration of a potassium-enriched diet together with elevation of plasma aldosterone reproduced the effects of chronic potassium adaptations observed in adrenal intact animals fed a high potassium diet (-0.76 ± 0.08 $\mu Eq/hr \cdot cm^2$). These results suggest that potassium adaptation in colon is caused by the independent effects of potassium loading and hyperaldosteronism acting together.

VII. CELLULAR MODEL OF POTASSIUM ADAPTATION

Figure 5 summarizes our findings regarding the mechanism of potassium adaption in rat colon. Chronic potassium loading enhances the rate of potassium secretion through changes in both apical and basolateral cell membranes. Potassium enters the cell in controls across the basolateral membrane by an active process mediated by the Na^+,K^+-ATPase pump and across the apical membrane by an absorptive potassium pump. Previous studies from this laboratory indicate that the absorptive pump is electroneutral and may represent a K:H exchange process (Foster *et al.*, 1984). In the steady state, cell potassium exits across both cell membranes according to the electrochemical gradients and membrane potassium conductances. Since the electrochemical gradients across the opposing cell membranes are approximately equal and potassium conductance in the basolateral membrane is probably greater than in the apical membrane, potassium movement toward the serosal solution is favored. This concept is consistent with the *in vitro* finding of net potassium absorption by control tissue (Foster *et al.*, 1984). Under *in vivo* conditions, however, a high luminal negativity favors potassium movement toward the mucosal solution via the paracellular shunt and across apical membrane.

In the potassium-adapted state, the rate of potassium uptake across the basolateral membrane is accelerated by an increase in the number of Na^+,K^+-ATPase sites, although the qualitative properties of the pump are unchanged, since the electrochemical gradient (E_{ec}) across the basolateral membrane is unaltered. However, since cellular potassium activity nearly doubles in the absence of a change in V_A, the electrochemical driving force acting on potassium diffusion across apical membrane increases by ~50%. In addition, potassium diffusion across the apical membrane is further enhanced by an increase in apical membrane potassium conductance, thus enhancing transfer of potassium toward the mucosal solution. Under *in vivo* conditions, net potassium secretion is further augmented by the rise in V_T due to hyperpolarization of the basolateral membrane, which increases potassium movement through the paracellular shunt pathway. Taken together, the increase in the rate of potassium uptake across the basolateral membrane, the rise in passive driving forces across the apical membrane, the increase in apical potassium conductance, and the increase in electrical gradient across the paracellular shunt pathway act synergistically to increase net potassium secretion.

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