MEDICAL GRAND ROUNDS

November 8, 1984

POTASSIUM DEFICIENCY: ITS COMPARATIVE EFFECTS ON SKELETAL MUSCLE AND THE HEART

JAMES P. KNOCHEL, M.D.

University of Texas Southwestern Medical School and
VA Medical Center, Dallas, Texas

POTASSIUM DEFICIENCY: ITS COMPARATIVE EFFECTS ON SKELETAL MUSCLE AND THE HEART

Potassium deficiency has been the subject of intensive investigation for many years. Some of its most important clinical manifestations center upon the heart and skeletal muscle. Studies on a variety of experimental animals including dogs, rats, guinea pigs and rabbits show that at least in early potassium deficiency, the deficit is borne almost exclusively by skeletal muscle (1-7). In contrast, other more vital organs, especially the heart (2,8) retain essentially normal potassium stores until the point at which the deficiency approaches the terminal state. In either situation, skeletal muscle tissue is consistently and uniformly deficient. An exception to the foregoing description of selective organ depletion of K may exist in the very young animal (8,9). Perhaps because of the added influence of growth, potassium deficiency always occurs more rapidly (tissue protoplasm synthesis without adequate potassium) and is generally more severe. Myocardial lesions may be produced easily in young animals (mice, rats) fed low potassium diets containing abundant sodium chloride (10) but are seldom seen in adult animals with potassium deficiency. Since a normal concentration of potassium ions in the heart and other vital organs such as the brain and the liver is a fundamental requirement for satisfactory function of those organs, it would appear that muscle functions as a reservoir destined to provide redistribution of its potassium ions when the total body is denied its normal requirements.

Until recently, little attention has been given to the significance of organ specific selectivity of potassium depletion. Information gained from techniques that permit characterization of mechanisms regulating intracellular potassium concentration have provided some explanation for this phenomenon which is not only of heuristic interest but also of clinical importance. Thus, as

will be pointed out, many of the adaptive responses to potassium deficiency mimic the responses to cardiac glycosides and may explain why hypokalemia increases the likelihood of developing digitalis toxicity.

Factors Responsible for Accumulation and Maintenance of Intracellular Potassium Ions

Three major forces are responsible for maintaining the usual potassium concentration in muscle and ventricular cytoplasm of about 150 mEq/L intracellular water (11). These include: (1) Donnan forces, (2) the relative impermeability of sodium as compared to potassium ions and (3) active sodium and potassium transport.

(1) In the classical Donnan model, a negatively charged nondiffusible macromolecule is placed in a cell and a solution of potassium chloride outside the cell. The cell membrane is freely diffusible to both potassium and chloride ions. At equilibrium, the concentration of potassium (more precisely, potassium activity) inside the cell is equal in chemical equivalents to the negative charges on the protein molecule plus that quantity in chemical equilibration with potassium ions outside the cell. Thus, Donnan forces establish a K^i/K^o gradient. The electrical force provided by the negative charge on the protein molecules that is required to exactly maintain the potassium gradient is the potassium equilibrium potential (E_k). The value of the potassium (equilibrium) potential can be estimated from the Nernst equation ($E_k = -61.5 + \log Ki/Ko$).

(2) Skeletal muscle cells and myocardial cells are more permeable to potassium than sodium ions. If one visualizes a model in which solutions of 0.15 molar sodium chloride and 0.15 molar potassium chloride are separated by a membrane having the same permeability characteristics of a muscle cell, potassium ions

will flow toward the sodium chloride side more rapidly than sodium flows toward the KCl side. A negative potential will be generated in the compartment that originally contained KCl. This potential will eventually reach a value that will precisely stop movement of potassium ions. Although the voltage generated is also a diffusion potential, it will not persist since both sodium and potassium ions will eventually come into chemical equilibrium and the charge on the membrane will be dissipated.

(3) To prevent dissipation of this charge and to maintain such a potential requires active transport. Thus, in the muscle cell, the ion pump is situated such that when sodium ions enter through their respective pores in the sarcolemmal membrane, an increase in Na concentration in the cytoplasm will activate the specific magnesium dependent, Na,K-ATPase which in turn will utilize the energy in ATP to actively pump sodium from the cell against its chemical gradient. In the process, potassium ions from the extracellular side of the sarcolemmal membrane will be pumped into the cytoplasm against its chemical gradient. Metabolic energy has been utilized. Because sodium ions are removed from the cell as rapidly as they enter, the cell is in effect impermeable to sodium ions. Thereby, the electrical potential difference generated by Donnan forces and by differential permeabilities of the membrane to sodium and potassium ions will be maintained by sodium transport. If the sodium, potassium exchange across the membrane were one-to-one, there would be no voltage generated. However, measurements of the Na:K exchange ratio in a variety of tissues, including cultured heart cells, indicate that the Na:K exchange ratio is 3:2. The net effect of this exchange is that three positively charged ions are removed from the cell while only two are permitted to enter and as a consequence, electronegativity is generated above that predicted by the potassium diffusion potential (12). If the Nernst equation is modified to include the permeability ratios for

sodium and potassium, as represented by the modified Goldman equation E_m = $-61.5 \log [K^i/(K_o + pNa^o)]$, the measured membrane potential (Em) may be predicted with reasonable accuracy unless the serum potassium concentration becomes significantly depressed (13).

Abundant evidence indicates that the membrane bound magnesium dependent, sodium and potassium activated ATPase is the enzymatic representation of the sodium-potassium pump not only in skeletal and cardiac muscle, but also in virtually every cell in the body. The bulk of available evidence indicates that digitalis glycosides and potassium ions compete for a common binding site on that portion of the enzyme oriented toward the outside of the cell membrane (fig 1). That portion of the enzyme oriented toward the cell interior is the binding

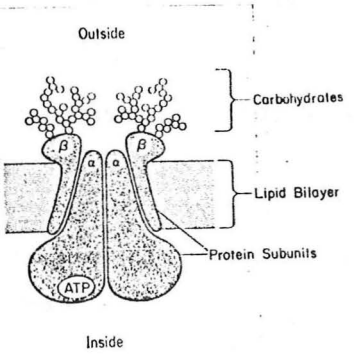


Figure 1. A Model of the Sodium-Potassium-ATPase. Each functional unit of the enzyme consists of two large (α) subunits that span the membrane and two smaller (β) glycoprotein subunits that are exposed on the outside of the cell. Each functional unit has one binding site for cardiac glycosides, one phosphorylation site, three binding sites for sodium and two binding sites for potassium.[13] How an apparently symmetric tetramer can have odd numbers of specific binding sites is an interesting problem. One possibility is that binding sites are formed at the interfaces of subunits. Another is that the two halves of the enzyme are in different but complementary conformations, and that when one half changes its conformation, the other half reciprocally changes too, so that the second conformation is the mirror image of the first.[12]

Sweadner and Goldin
NEJM 302:777, 1980

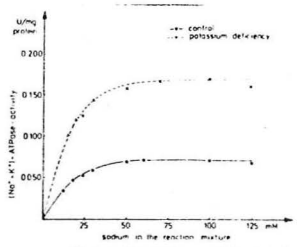
site for sodium ions and specific antibodies against the ATPase molecule (14). An excellent review on the interaction of cardiac glycosides with Na+, K acti-

vated ATPase was published within the past month by Hansen (15).

Responses of the Sodium Pump to Potassium Deficiency and Digitalis Glycosides

Within five days to three weeks after administering a potassium deficient diet to experimental animals, there follows an increase in the net number of ouabain-binding sites in the heart (5,16) and either an increase or no detectable change in density of these sites in the brain (6) kidney (5,17) or red cells (18). In contrast, at the same time there occurs a sharp decrease in the number of ouabain binding sites in skeletal muscle. That this response has been consistently more pronounced in white muscle fibers than red muscle fibers might be related to the higher K concentration and resting membrane potential of white muscle fibers (19-22). Presumably, these two features usually predict a greater density of pump units.

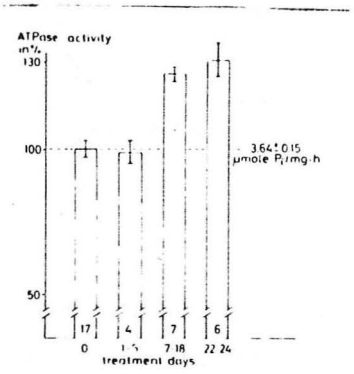
To characterize the effects of K-deficiency on the heart, Erdmann et al (16) prepared isolated membranes from samples of myocardium from normal and potassium deficient animals. Ventricular sarcolemmal ATPase from normal animals measured 0.075 units/mg (1 unit = the amount of enzyme catalyzing the conversion of 1 micromole of substrate per minute at 37°C) (fig. 2). In the K-deficient



$(Na^+ + K^+)$-ATPase activity in relation to the concentration of Na^+ in the presence of 10 mM KCl, 2 mM ATP, 3 mM $MgCl_2$, 67 mM imidazole HCl, pH 7.4, 37°. Incubation was carried out for 60 min. The $(Na^+ + K^+)$ ATPase data are the results of four determinations at each concentration. The heart muscle extracts of five control animals and five potassium deficient animals were sampled.

animals, serum potassium fell from an average value of 4.3 to 2.5 mEq/L. Ventricular ATPase activity in potassium deficient animals measured 0.172 units/mg, representing a net increase of 130%. The ouabain-inhibited enzyme represented 16.6% of total ATPase activity in the control animals and 28.3% in the potassium deficient animals. They also measured the effect of various concentrations of sodium, potassium and Ouabain on the apparent activity of the enzyme. Their results indicated a net increase in Na,K ATPase activity in the potassium deficient animals. Since animals fed potassium deficient diets usually become anorectic and lose weight, control measurements of ATPase activity were also conducted on animals that were deliberately underfed. The heart muscle extracts of these animals showed identical values to those of the control animals.

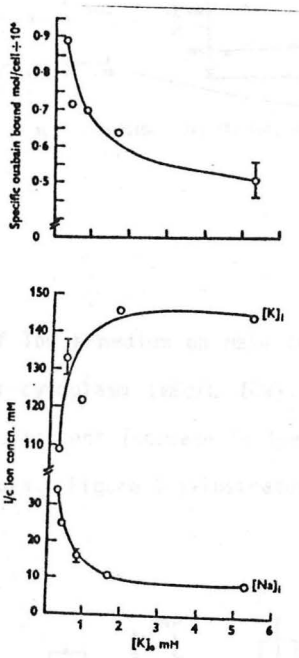
Bluschke and associates (5) also demonstrated a net increase in Na, K-ATPase activity in male guinea pigs fed a potassium deficient diet for 12 days. Serum potassium had fallen from an average value of 5.1 to 3.4 mEq/L. The increase in ATPase activity was limited to ventricular muscle (fig. 3).



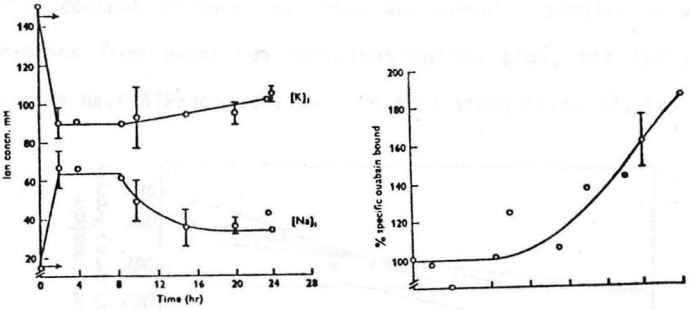
BLUSCHKE, V. ET AL. EUROP. J. PHARMACOL. 37:189-191, 1976

Their was no change in specific activity of the ATPase enzyme in kidney or brain.

Boardman, Huett, Lamb, Newton and Polson (23) examined genetic control of sodium pump density in Hela cells grown under conditions of low potassium concentration. Following culture in a low potassium medium for 24 hours (fig. 4),

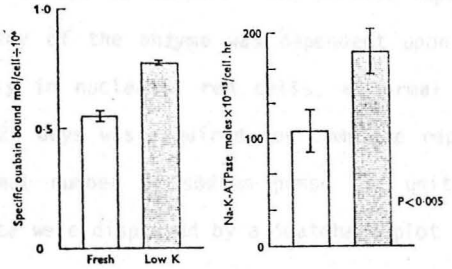


these cells showed an increased sodium concentration, a decreased potassium concentration, increased Ouabain binding and increased Na-K ATPase activity. Their data suggested that the ATPase response both in activity and quantity was primarily a response to increased intracellular sodium concentration. Figure 5

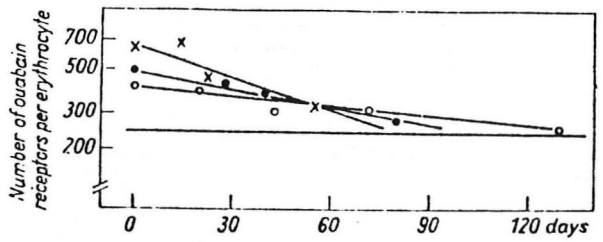


BOARDMAN, L. ET AL. J. PHYSIOL. 241:771-794, 1974

shows the time course of low K medium on Hela cells. The left panel shows Na and K concentrations in cytoplasm (mEq/L ICW). Specific ouabain binding is shown on the right. The percent increase in the latter was nearly 200% in the low K medium after 28 hours. Figure 6 illustrates effect of growth of Hela

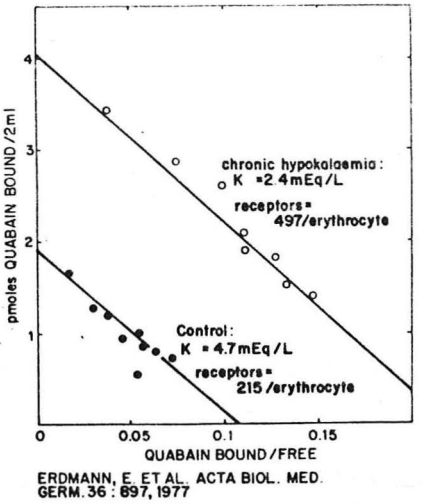


Erdmann and Krawietz subsequently conducted studies on erythrocytes from patients with chronic hypokalemia (18). It is noteworthy that red cell potassium content in these patients was normal. Similar to ventricular muscle preparations from potassium deficient guinea pigs, red cells also show a net increase in Na,K-ATPase activity. In this study (fig. 7), the density of



ERDMANN, E. ET AL. ACTA BIOL. MED. GERM. 36:879-883, 1977

pump units was also estimated on the basis of Ouabain binding sites per mL of erythrocyte membranes. In normal humans, this value was 235 $\pm$ 48. In patients with chronic hypokalemia (serum K 2.8 mEq/L), the value was 476 $\pm$ 185. Following correction of hypokalemia, 75 to 135 days were required for recovery of pump density values to normal. The obvious implication from these studies was that recovery of the enzyme was dependent upon protein synthesis. Since this occurs only in nucleated red cells, a normal red cell survival time of approximately 120 days was required for complete replacement by new cells containing an normal number of sodium pumps per unit cell membrane. When the experimental data were displayed by a Scatchard plot (fig. 8), there was iden-

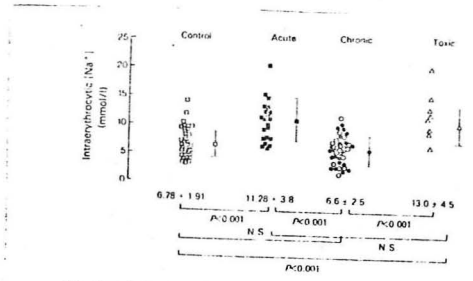


ERDMANN, E. ET AL. ACTA BIOL. MED. GERM. 36 : 897, 1977

tical affinity of the receptor for the ligand at all concentrations. This implied the existence of only one type of receptor, and that the number of receptors per unit membrane mass had increased as the result of potassium deficiency. Acute hypokalemia in patients with vomiting and diarrhea had no effect on specific ouabain binding sites.

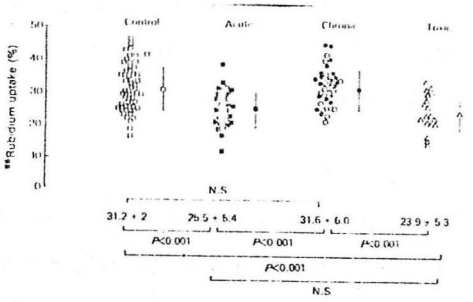
Chan and Sanslone (24) studied rats fed a K-deficient diet for 10 weeks. Red cell Na, K-ATPase doubled within this period. As evidence that the adaptation involved only this enzyme, they also measured another membrane-bound enzyme, acetylcholine esterase, which did not change. Following return to a normal K intake, 5 weeks elapsed before red cell ATPase returned to normal.

Studies have been conducted on red cells to characterize cardiac glycoside receptor sites, rubidium uptake as an inferential index of potassium transport and intracellular sodium concentration in patients during the early phase of digoxin therapy and again after two months of chronic treatment (25). The characteristic response within the first few days after administration of digoxin is a reduction of rubidium uptake, elevation of intracellular sodium concentration, and reduction of digoxin binding to red cell membranes. Figure 9



Comparison of intraerythrocytic sodium concentrations in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml

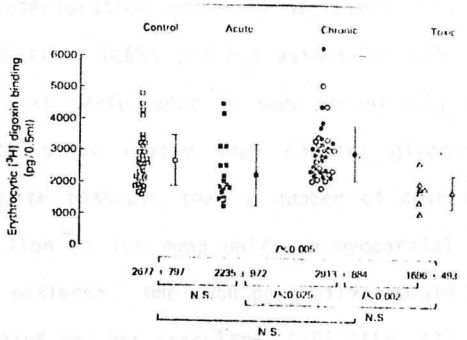
shows individual as well as mean values for red cell sodium concentration before treatment, during the acute phase, following chronic administration and with digoxin toxicity. It is of interest that the sodium concentrations in the control and chronic groups were not different, implying net recovery of sodium transport. Similarly, values in those studied acutely and those with digitalis toxicity were also similar, implying that digitalis poisoning impairs transport although earlier adaptations had occurred. Figure 10 shows similar rela-



Comparison of ^{86}Rb uptake in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml.

tionships for rubidium uptake as an index of potassium transport. The reduction of rubidium transport was similar in acutely treated patients as well as those

treated to the point of toxicity. Erythrocyte digoxin binding (fig. 11) did not

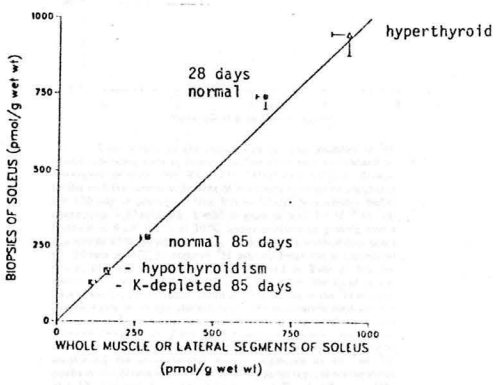


Comparison of [^{3}H]-digoxin binding in the four groups of patients. In the chronic group the open circles refer to patients with plasma digoxin concentrations less than 0.8 ng/ml

change significantly during the initial treatment period. Although there was a fair amount of scatter in the results, digoxin binding in patients with digoxin toxicity was significantly less than values in the control subjects. Increased digoxin binding in erythrocytes following chronic treatment suggests that erythrocytes formed during treatment may possess a increased number of available cation pumping sites as a result of being exposed to digoxin during erythropoiesis. At least in the study by Vaughn and Cook (26) a prolonged elevation of the internal sodium concentration was a requirement for adaptation to occur.

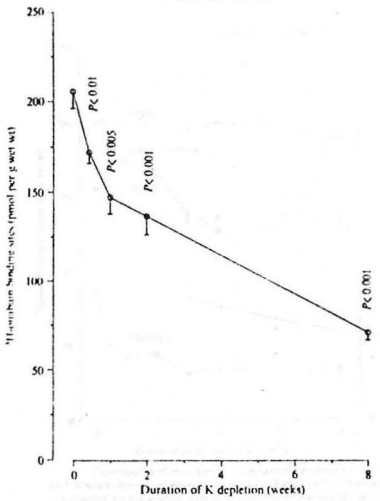
The inotropic effect of digitalis appears to result from the sequence of Na, K-ATPase inhibition, decreased sodium efflux, increased intracellular sodium concentration, reduced Na:Ca cellular exchange, and increased contractility as a result of cytosolic calcium accumulation (27). If the myocardium of man increases ouabain binding sites in response to digitalis, then one can explain the observations by some investigators that digitalis therapy is no more than transiently beneficial (28-33). A number of investigators were unable to detect

any apparent clinical effect of digoxin after one month of administration or, on the other hand, no deterioration following digitoxin withdrawal. Fonrose et al 1974 (34) and in addition, Dobbs and his associates (35) did find evidence of deterioration of cardiac performance in some chronically treated patients with cardiac failure with sinus rhythm when cardiac glycosides were withdrawn. However, it seems quite possible that a number of coincidental factors could prevent upward induction of ion pump units in myocardial cells as a result of digitalis in certain patients. One good possibility would be hypothyroidism, as demonstrated by Norgaard and his associates (36) (fig. 12).



NORGAARD, A. ET AL. BIOCHEM. BIOPHYS. RES. COMM. 111:319-325, 1983

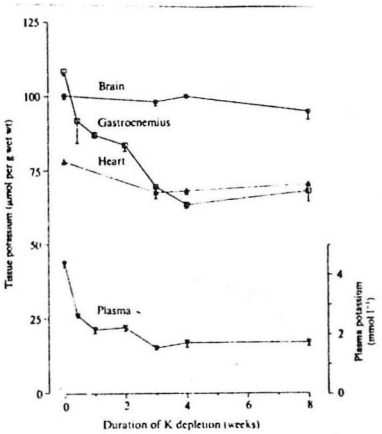
The foregoing effects of potassium deficiency on pump activity in myocardium, red cells, and cultured cell lines (23) stand in sharp contrast to those observed in skeletal muscle. Norgaard and his associates (6) examined 3H-Quabain binding and ^{42}K uptake in soleus and extensor digitorum longus (EDL) muscle from either rats or mice during potassium deficiency induced by administering a potassium free diet, a diuretic or a potassium-binding resin. Potassium deficiency decreased the number of binding sites as much as 78% (fig. 13). The defect was reversible by repletion of potassium. Sodium-potassium



Time course of the changes in the total number of ^{3}H ouabain binding sites in soleus muscles from rats maintained on potassium-deficient diet. Rats (210–240 g) were killed by decapitation and the lateral segments of their soleus muscles incubated for 120 min in potassium free Krebs–Ringer bicarbonate buffer containing 1.27 mM Ca, 5 mM D-glucose and 2×10^{-6} M ^{3}H-ouabain (0.6 μCi ml^{-1}) at 30 °C under continuous gassing with a mixture of 95% O_2 and 5% CO_2. Muscles were washed four times for 30 min at 0 °C to remove ^{3}H activity from the extracellular space, blotted, weighed and homogenized in 2 ml of 5% trichloroacetic acid. After centrifugation, 1 ml of the clear supernatant was taken for liquid scintillation counting of the ^{3}H activity. On the basis of the specific activity of the incubation medium, the amount of ^{3}H activity retained following the cold wash was expressed as pmol per g wet wt. A minor correction for nonspecific retention of ^{3}H activity was based on measurements performed by incubating the contralateral muscle segments at 10^{-3} M ^{3}H-ouabain (for details see refs 9–11). Each point represents the mean of 4–12 observations with bars denoting s.e. The significance of the difference between the results obtained using age-matched controls maintained on normal diet and those of the K-depleted rats is given above each point.

transport-mediated 42K uptake in skeletal muscle was also decreased by potassium deficiency. In both rats and mice, maintenance on a potassium deficient diet

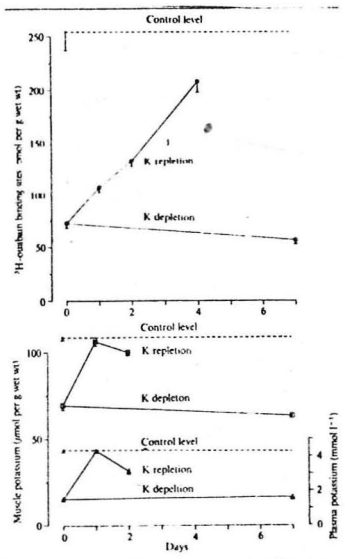
induced a rapid reduction in potassium content of plasma and skeletal muscle, but essentially no change in heart or brain (fig. 14). The values in muscle



Time course of the changes in potassium contents of tissues and plasma during potassium-depletion. Rats (110–240 g) were maintained on potassium-deficient diet (Altromin) for the periods indicated. Blood samples were withdrawn from the carotid artery under light ether anaesthesia, collected into glass tubes wet with a heparin solution (30 IU ml $^{-1}$) and centrifuged. Tissue samples were taken immediately after decapitation, homogenized in 5% trichloroacetic acid and centrifuged. The potassium and sodium contents of the plasma and the trichloroacetic acid extracts were determined by flame photometry using a Radiometer FLM 3 flame photometer with lithium as internal standard. Each point represents the mean of 2–14 observations with bars denoting s.e., where this exceeds the size of symbols.

and plasma reached their nadir after 3 to 4 weeks on the diet and remained stable for an additional 4 weeks of the experiment. This characteristic pattern of potassium deficiency had also been noted in our own experiments on dogs fed potassium deficient diets (13,37). Thus, depletion of potassium is characterized by the rapid appearance of hypokalemia and a fairly rapid decline of muscle potassium content to values of approximately one-half of normal. After a period of about 2 weeks, depending upon salt intake and mineralocorticoid administration, both of these values tend to stabilize for many additional weeks. For example, it is not uncommon to observe potassium losses amounting to 15 to 20% of total body stores during the first 12 to 15 days of potassium depletion. Thereafter, an additional 20 to 30 days may pass before loss of an

additional 5% occurs. The gradual reduction in the rate at which potassium is lost during potassium deprivation thus appears to be a characteristic of several species. Norgaard and his co-workers also examined ^{3}H-ouabain binding, muscle and serum K during K-repletion in the same animals (fig. 15). Each of the three parameters showed a sharp recovery (6).



Time course of changes in the total number of ^{3}H-ouabain binding sites and the potassium contents of plasma and gastrocnemius during potassium repletion. Rats (110–170 g) were maintained on potassium-deficient diet for 3 weeks. One group of animals were kept on this diet for another week, others were given KCl (16 mmol per kg per day) for either 1, 2 or 4 days. Potassium contents and ^{3}H-ouabain binding were determined as described in Figs 1 and 2. Each point represents the mean of 2–16 observations with bars denoting s.e. Dashed lines indicate control levels measured in rats on normal diet.

Studying rats, Williams, Withrow and Woodbury (38), examined the effects of ouabain on the resting membrane potential of skeletal muscle, intracellular and extracellular electrolytes. The measured muscle resting potential difference decreased from -90 mV to -65 mV with ouabain which was predictable from measurements of intracellular and extracellular electrolytes. The effects of ouabain also include a rise in potassium and a fall in sodium concentration in plasma, a

rise in intracellular sodium and chloride and a fall in potassium concentration. Evidence such as this is perhaps the most valid indication that ouabain acts upon skeletal muscle ATPase and lends credance to the studies published by Norgaard (6) and Clausen (38a).

One must be extremely careful about the assumption that changes in specific ouabain binding of muscle following such interventions as potassium deficiency has truly quantitated a change in the number of receptors. For example, it is known that hypokalemia may be responsible for increased peripheral vascular tone and increased peripheral resistance to blood flow in the vascular bed of skeletal muscle (39). Thus, simply injecting a dose of labeled ouabain into an intact animal and subsequently measuring its accumulation in skeletal muscle might well lead to erroneous interpretations. For example, we know that even normal muscle tissue is poorly perfused at rest. Studies such as those performed by Norgaard and Clausen (6,38) employ intraperitoneal injection of labeled ouabain followed by collection of muscle samples. There has been no attempt to open up the capillary bed or on the other hand, to induce muscle cell hyperemia that may occur, for example, during exercise. Nevertheless, following injection of labeled ouabain, those investigators compared *in vivo* uptake to *in vitro* uptake of the label by skeletal muscle membranes and showed that there was no difference between the two values (Table). Therefore, we can assume that *in*

[^{3}H] OUABAIN BINDING SITES IN RAT SOLEUS*
(PMOL/G WET WEIGHT)

	IN VITRO (N)	IN VIVO (N)	
UNTREATED	237 ± 12 (8)	247 ± 12 (12)	$P < 0.001$
K-DEPLETED**	95 ± 4 (8)	92 ± 11 (8)	$P < 0.001$

* CLAUSEN ET AL. J. PHYSIOL. 333:367, 1982.
** K-FREE DIET 3 WEEKS

vivo, labeled ouabain is specifically distributed to and becomes bound to skeletal muscle receptors, and by all evidence measures the density of pump enzyme in sarcolemmal membranes.

Cameron and his associates (40) have performed several studies on muscle composition and sodium pump density on rabbits during induction and recovery from K deficiency. During repletion, muscle recovered its potassium content in one week. However, during the same time, myocardial K showed a rebound to values above normal. They ascribed this event to the upward regulation of sodium pump units in the heart but not skeletal muscle. Continued studies by the same workers (41) showed an apparent change in the kinetics of Na pump activation by K. Vmax of the partially purified enzyme from the heart rose substantially during K deficiency. In addition, the affinity of the enzyme for K also increased. The Km fell from control values of 1.04 $\pm$ 0.12 mM to 0.62 $\pm$ 0.09 in the depleted group ($p < 0.001$). They calculated that this reduction in Km would increase activity of the enzyme 15% at the usual plasma K concentration of 20 mEq/L in deficient animals. Unfortunately, these authors have not published their data in a reviewed journal and I know of no other investigations of Na, K-ATPase kinetics in potassium deficiency.

The observation that during the course of depletion, the rate of potassium loss eventually slows down may also have a clinical corollary in patients medicated with potassium-wasting diuretics. Thus, it has been noted that in some of these patients, hypokalemia and a net loss of potassium may occur early during the course of diuretic administration. However, after a period of weeks or months, serum potassium tends to return toward normal or stabilize at only slightly low values and at this point, potassium wasting can be demonstrated only with difficulty.

Akaike (42) noted that potassium deficient diets caused a decrease in potassium concentration in blood plasma and skeletal muscles but not in cerebrospinal fluid or brain tissue. During the first 4 to 5 weeks of potassium deficiency, decreases in rat muscle potassium are not compensated by equivalent increases in muscle sodium. However, during further hypokalemia, the ratio of the number of sodium ions entering the muscle to that of potassium ions coming out approaches one. The plasma potassium concentration in the hypokalemic rats decreases from a level of about 4.6 mmoles to about 1.6 mmoles, a value that is sufficiently high to maintain activity of the sodium-potassium pump in muscle (recall that the "K_m" for potassium activation of Na,K-ATPase is about 1 mmole). Furthermore, when muscles from hypokalemic rats are excised and placed in Kreb's solution at 37°C, the intracellular sodium concentration is promptly reduced and the intracellular K concentration is corrected, even though the external potassium concentration remains as low as 1 mmole. He concluded that in hypokalemic rats, the Na-K pump is obviously inhibited, however the high intracellular sodium and low intracellular potassium of muscle in situ cannot be attributed to pump inhibition by plasma hypokalemia. (Usually, the pump begins to fail when plasma K falls below 1.0 -1.5 mEq/L). Therefore there must be an additional mechanism by which activity of the sodium-potassium pump in muscle of hypokalemic rats becomes suppressed <u>in vivo</u>. Based upon the information that adrenergic receptors modulate serum potassium concentration, Akaike considered the possibility that increased noradrenergic tone might be the factor responsible for the eventual characteristic finding in potassium deficiency that serum potassium and muscle potassium concentrations tend to reach a plateau. As an intervention to reduce noradrenergic tone, he sectioned the brain, spinal cord or peripheral nerves. After denervation, the soleus muscle promptly restored its sodium and potassium concentrations to values close to those of soleus muscles from normal rats. The half time for recovery of these internal cations

was only 20 minutes. Within a period of 6 hours, ionic composition of the muscle cells had been essentially restored to normal although serum potassium remained at very low levels. The recovery was inhibited by ouabain thereby implicating the sodium-potassium pump. Akaike then demonstrated that administration of the α-adrenergic blocking agent, phentolamine, exerted the identical effect on muscle potassium concentration recovery despite ongoing hypokalemia. He observed a similar response to dibenamine and phenoxybenzamine. In contrast, administration of propranolol, a non-selective β-adrenoreceptor antagonist, produced no changes in either sodium or potassium contents of the skeletal muscle cells. Akaike suggested that the endogenous transmitter norepinephrine might be responsible for the eventual stabilization of potassium uptake in potassium deficient animals and indeed, is probably responsible for continued suppression of pump activity so that serum potassium levels may be maintained at a level sufficient to maintain life. It is interesting that others have demonstrated increased levels of plasma catecholamines in potassium deficient animals (43), increased norepinephrine content in secretory vesicles of nerve endings (44), and decreased norepinephrine vasoresponsiveness in K-deficient rats (45) and patients (46).

Clausen, Kjeldsen and Norgaard (47a) claimed that they were unable to confirm certain elements of Akaike's observation that phentolamine caused K uptake in potassium deficient muscle. However, the dose of phentolamine employed by these investigators was substantially less than that employed by Akaike. Clausen and his associates also claimed that they could induce correction of muscle potassium content by immobilization of an extremity by a variety of means. Unfortunately, they did not report serum potassium values or acknowledge the fact that total immobilization of a limb in a plaster cast or inactivation of a muscle by tenotomy could conceivably facilitate an atrophic or catabolic

response in that particular tissue, liberate its potassium stores and thereby restore serum potassium values to normal. This response in turn would tend to correct potassium values for other muscles not immobilized. It is noteworthy that Clausen and his associates described a decrease in the number of 3H-ouabain binding sites ranging from 8 to 13% following one week of denervation. Since a decrease in the number of ouabain binding sites corresponds to a decrease in ATPase activity, the latter findings may be a mirror image of our own findings that intensive physical training of skeletal muscle causes an increase in sodium transport ATPase activity (47a).

The Interrelation of Potassium Deficiency, Age, Pump Density and Digitalis Toxicity

That hypokalemia produced by potassium deficiency may reduce the toxic threshold to digitalis has been clearly appreciated for many years. One of the most lucid and comprehensive reviews on this subject was published by Lown, Black and Moore in 1960 (47). At that time, information was just beginning to emerge suggesting that the mechanism of action of cardiac glycosides might be to inhibit Na, K-ATPase, based upon studies conducted on the enzyme from other tissues (48,49).

The interactions of potassium and the toxic effects of digitalis were clearly appreciated. For example, Lown and his associates (50) studying trained dogs, observed that when serum potassium was maintained between 7 and 8 mEq/L by hemodialysis, the dose of digitalis required for a toxic effect was increased approximately 240%. Of interest, Garb and Venturi (51) showed that while potassium may antagonize the toxic effects of digitalis, it would not block the increase in contractility resulting from the addition of ouabain. Conversely, Lown and his associates showed that when 5 to 10% of a dog's total body

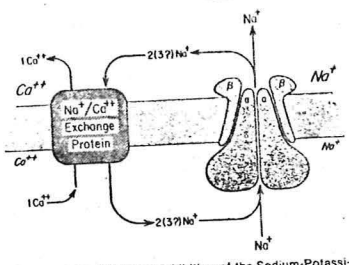
potassium was removed and the serum concentration lowered to 2 mEq/L, only 40% of the usual toxic dose of digitalis was required to produce ventricular tachycardia. Finally, alteration of the threshold for digitalis toxicity is not necessarily the result of potassium deficiency or net retention of potassium ions by the body. Thus, digitalis intoxication may appear rapidly if serum potassium is reduced without changing total body potassium by the administration of sodium chloride, sodium bicarbonate, glucose or insulin. It has been shown (52,53) that in the critically digitalized patient, induction of sudden hypokalemia by oral administration of carbohydrates or intravenous administration of glucose or insulin may precipitate ventricular premature beats or ventricular tachycardia. Francis and his associates (54) showed that acute hypokalemia in dogs induced by glucose-insulin increased ^{3}H-digoxin uptake by the left ventricle but not skeletal muscle.

Based upon the aforementioned studies by Norgaard and his associates (6) demonstrating that specific 3H-ouabain binding in skeletal muscle was markedly reduced in potassium deficient animals, it was postulated that this characteristic may favor redistribution of digitalis glycosides from the periphery to the heart and may explain the increased likelihood of digitalis toxicity as the result of potassium deficiency. Since skeletal muscle represents the largest pool for cardiac glycoside binding, the fractional binding potential for a given dose of cardiac glycoside by the heart would be enormously increased in animals (and presumably man) as a result of potassium deficiency.

An additional important observation reported in the study by Norgaard et al (6) and Kjeldsen (55) and his co-workers was a sharp reduction in the ability of skeletal muscle cells to take up potassium ions as a result of potassium deficiency. Control animals showed a potassium uptake of 0.377 micromoles per g wet-weight per minute. In animals that were potassium deficient for a period

of 3 weeks, potassium uptake was reduced to 0.154 micromoles/g/wet-weight per minute, a difference that was highly significant. Even after potassium supplements were given for one day in large doses, the ability to take up potassium remained abnormally low. However, when potassium uptake was expressed as the number of ions taken up per ouabain binding site per minute, the value remained normal for the period of depletion and early repletion. This observation suggests that potassium uptake by skeletal muscle is principally a function of pump enzyme units per mass of muscle tissue. These observations suggest at least two points of potential clinical importance. First, it has been observed that some patients with established potassium deficiency and frank hypokalemia may rapidly become hyperkalemic following administration of ordinary doses of potassium salts intravenously. This unexpected response might be the result of decreased pump unit density in skeletal muscle and explains variation in potassium tolerance among hypokalemic patients. Second, correction of hypokalemia in a potassium-deficient patient does not assure protection against digitalis toxicity. The foregoing studies on animals suggest that 24-48 hours may be required for reconstitution of pump sites in skeletal muscle.

How might one integrate the foregoing information with that presented earlier concerning the effect of potassium deficiency on the density of pump sites in the heart and skeletal muscle? Current evidence suggests that the increased myocardial contractility that occurs following digitalis therapy is related to a slight increase of cytosolic calcium concentration in the myocardial cell (56,57). Suppression of ATPase activity by the cardiac glycoside reduces sodium transport from the cell. This action has two specific effects. The first is an increased concentration of sodium ions in the cytosol; the second is a reduction in the resting membrane potential of the cell. These two factors directly influence the sodium-calcium exchange that is also located in the sarcolemmal membrane (fig 16). Normally, active transport of sodium from



A Model of How Inhibition of the Sodium-Potassium-ATPase by Digitalis Can Affect Calcium Levels in Cardiac Muscle Cells.

Normally the sodium-potassium-ATPase establishes sodium gradients that are the driving force for calcium efflux by a separate sodium-calcium exchange system. Some of the sodium pumped out by the sodium-potassium-ATPase reenters the cell through the exchange system, producing coupled net efflux of an equivalent amount of positive charge in the form of calcium. Such an exchange system is present also in nerves, where there is evidence that each calcium ion may be coupled to the transport of three sodium ions rather than two sodium ions as originally proposed for cardiac muscle. Inhibition of the sodium-potassium-ATPase reduces the magnitude of sodium pumped out, thus reducing the driving force for calcium efflux through the sodium-calcium exchange mechanism. This inhibition leads to increased intracellular calcium levels and, consequently, increased muscle contractility. The model is an example of the part that the sodium-potassium-ATPase can play in regulating the level of other intracellular molecules besides sodium and potassium.

Sweadner and Goldin
NEJM 302, 1980

the cell increases the extracellular to intracellular Na^+ concentration gradient across the myocardial cell. The increased gradient thus becomes a chemical force tending to drive sodium ions back into the cell. Secondly, as sodium is actively transported from the cell by the action of ATPase, the electrogenic effect of the pump increases the usual electronegativity inside the cell. This force also attracts positively charged sodium ions from outside the cell. Thus, forces promoting the inward traffic of sodium activate the sodium-calcium exchanger that in turn facilitates outward movement of calcium ions. This system regulates in a fine way the normally very low concentration of free calcium ions in the cytosol. Accordingly, even slight inhibition of the ATPase pump by digitalis glycosides permits accumulation of sodium ions in the cell, which in turn decreases the chemical gradient of sodium ions across the cell, and the decreased electrogenic sodium transport from the cell reduces the

membrane potential. Finally, the electrical forces tending to pull sodium ions back into the cell are weakened. The result is an accumulation of calcium ions so that now the myocardial contractile proteins respond more briskly, thereby accounting for increased contractility of the heart following digitalis therapy. Since both the therapeutic effect and toxicity to digitalis glycosides are dose related, the effect of hypokalemia to substantially reduce the enormous number of binding sites for digitalis in skeletal muscle and simultaneously to increase the number of binding sites in cardiac muscle sarcolemmal membranes readily explains why digitalis toxicity is more likely to occur in hypokalemia with conventional doses of digitalis. Thus, the simple explanation seems to be that more of the drug will be available to interact with receptors in the heart thereby increasing a likelihood of toxicity.

Some of the observations made on the density of ouabain receptors and myocardial and skeletal muscle cells by Norgaard and Clausen may also explain why the likelihood of digitalis toxicity increases with age (38). They observed that the net number of ouabain binding sites in soleus muscle of rats decreased by 58% between the age of 28 and 85 days. Following the injection of labeled ouabain, the 85 day old rats showed a more pronounced and sustained rise of plasma 3H-ouabain activity which presumably reflects the reduced ability to bind ouabain in skeletal muscle receptors. It is of interest that muscle cells of young rats (and presumably the young of other species) have a high sodium content and a low membrane potential that gradually become normal by the age of about 3 months (58). A number of studies have shown that certain factors that cause increased sodium permeability of cells, whether it be immaturity (58) malnutrition (59), or early thyrotoxicosis (60), will be associated with an adaptive increase in net number of ouabain binding sites in the sarcolemmal membrane of skeletal muscle. The practical implication is that as animals and

presumably humans merge from infancy to childhood, intracellular sodium concentration declines and in association, the net quantity of pump units decline thus increasing the likelihood for digitalis toxicity. If hypokalemia is superimposed, which also decreases the net number of ATPase enzyme units in skeletal muscle, then the susceptibility to the effects of digitalis increases. Of interest, experimental administration of digitoxin for 15 days yielded an increase of ATPase activity in heart muscle to almost an identical degree with that produced by potassium deficiency (38). Similar to K deficiency, there was no change in kidney or brain ATPase activity with digitoxin administration. The obvious gap in our information concerns the reason why both digitalis and potassium deficiency selectively reduce the density of sodium pumps in skeletal muscle but not other tissues.

The Role of Intracellular Calcium in Potassium Deficiency

It is well known that calcium opposes the electrical effects of potassium on excitable tissues and also that hypercalcemia increases the propensity toward digitalis toxicity. It has been known for many years that if the level of calcium in the perfusate is increased when studying the isolated frog heart preparation, systole becomes more vigorous. This effect of calcium is opposed by raising the concentration of potassium. Clinically, profound hypocalcemia has been described as a cause of impaired myocardial contractility with congestive heart failure.

It was stated earlier that increase of potassium concentration may prevent digitalis-induced arrhythmias while a decrease of potassium may sensitize the heart to such disorders. This same relationship holds if calcium is substituted for digitalis. In the isolated rabbit heart, ventricular fibrillation can be initiated by injection of calcium or by perfusion with a potassium free solution

(61). Calcium injection does not result in fibrillation when the potassium concentration exceeds 2.3 mEq/L. Similarly, a potassium free solution does not induce ventricular fibrillation if calcium concentration is less than 3.5 mEq/L. A relationship between calcium and digitalis has also been observed on the transmembrane potentials of individual myocardial fibers (62). The usual shortening of the transmembrane action potential that occurs within three hours after digitalization will be decreased to one hour if the extracellular calcium concentration is doubled. If calcium is excluded from the perfusing fluid, digitalis effects do not appear. This observation concurs with the evidence that a major effect of digitalis is facilitation of calcium entry into the myoplasm.

It has been shown that experimental potassium deficiency in dogs is associated with an increase of skeletal muscle calcium content. Unpublished results from our own laboratory have shown that when skeletal muscle potassium content was reduced from 44 to 28 mEq/100 g fat free dry weight, total muscle calcium content rose from 1.8 to 3.9 mEq/100 g FFDW. Unfortunately, measurements of total calcium content tell us very little since muscle calcium is normally compartmentalized in sarcoplasmic reticulum and mitochondria.

Ledwoch and his associates (63) studied the influence of chronic potassium deficiency in rabbits maintained for an average of 28 days on a potassium deficient diet. Serum potassium showed a decline from 4.9 to 2.2 mEq/L while serum sodium concentration rose from an average value of 140.2 to 151 mEq/L. Measurement of myocardial potassium content at 28 days showed that this value declined from an average of 59.2 mmoles/kg heart tissue to 51.9 mmoles. These investigators found no change in the contents of sodium, calcium or magnesium in myocardial tissue. However, mitochondria isolated from potassium deficient ventricular myocardium showed an increased state of respiration and in addition,

an increase in the rate of calcium uptake. Passive calcium binding to isolated cardiac mitochondria was significantly higher. These data were interpreted to suggest that mitochondria are able to store and take up more calcium in potassium deficiency. Presumably, mitochondrial Ca^{++}-ATPase was induced by an elevated cytosolic Ca concentration. The mitochondrial phospholipid pattern showed a depression of phosphatidylcholine and a relative increase in the content of phosphatidylethanolamine. The precise role that these alterations play in the propensity for digitalis toxicity in potassium deficiency is unknown.

Summary

1. Short-term potassium depletion causes a selective deficiency of potassium in skeletal muscle.

2. In the heart, either potassium-deficiency or cardiac glycosides independently cause an upward regulation of sodium:potassium transport ATPase.

3. In skeletal muscle, in contrast to the heart; potassium deficiency causes a frank reduction of sodium pumps.

4. Since cardiac glycosides bind to receptors on the sodium-potassium pump, the decrease in pump density in K deficiency results in decreased glycoside binding in muscle. With a given dose of digoxin, muscle uptake is less, plasma levels and myocardial uptake are higher and digoxin poisoning is more apt to occur.

5. For any given method of experimental K depletion, the pace at which potassium deficiency occurs slows down spontaneously at the 4th or 5th week. Evidence suggests that this is the result of Na-pump inhibition in skeletal muscle. Interruption of α-adrenergic activity by denervation or by pharmacologic means is promptly followed by reconstitution of skeletal muscle cell potassium despite sustained hypokalemia. Thus, it has been proposed that the apparent increase of norepinephrine secretion may be at least one factor responsible for continued K loss from muscle to provide K for vital organ functions.

6. The capacity for potassium uptake by skeletal muscle is reduced in K-deficient animals. This may not only explain the occasional occurrence of unexpected hyperkalemia during conventional KCl administration to patients with hypokalemia, but also serves notice that total body K deficits can not always be rapidly replaced.

7. Finally, there is no explanation why potassium deficiency or digitalis therapy exert opposite effects on Na-pumps in skeletal muscle and the heart. This would obviously be an important and practical field for investigation.

REFERENCES

1. Bahler, R.C., and Rakita, L.: Cardiovascular Function in Potassium-Depleted Dogs. Am. Heart J. 81:650-657, 1971.

2. Prioreschi, P.: Role of Potassium in the Pathogenesis of the "Electrolyte-Steroid-Cardiopathy with Necrosis". Circ. Res. 10:782-785, 1962.

3. Tucker, V.L., Hanna, H., Kaiser, C.J., and Darrow, D.C.: Cardiac Necrosis Accompanying Potassium Deficiency and Administration of Corticosteroids. Circ. Res. 13:420-431, 1963.

4. Zeeman, S., Hirsch, S., and Bellet, S.: The Effect of Potassium Depletion Induced by Desoxycorticosterone Acetate on the Lethal Dose of Lanatoside C in Dogs: Relationship of Plasma Levels, Skeletal and Cardiac Muscle Potassium Content to the Lethal Dose. Am. J. Med. Sci. 277:65-73, 1954.

5. Bluschke, V., Bonn, R., and Greeff, K.: Increase in the $(Na^+ + K^+)$-ATPase Activity in Heart Muscle After Chronic Treatment with Digitoxin or Potassium Deficient Diet. Europ. J. Pharmacol. 37:189-191, 1976.

6. Norgaard, A., Kjeldsen, K., and Clausen, T.: Potassium Depletion Decreases the Number of ^{3}H-Ouabain Binding Sites and the Active Na-K Transport in Skeletal Muscle. Nature 293:739-741, 1981.

7. Knochel, J.P., Foley, F.D., Jr., and Lipscomb, K.: High Resting Cardiac Output with Exercise-Induced Pulmonary Edema in the Conscious, Potassium-Deficient Dog. Min. Elect. Metab. 1:336-344, 1978.

8. Gunning, J.F., Harrison, C.E., Jr., and Coleman, H.N., III: The Effects of Chronic Potassium Deficiency on Myocardial Contractility and Oxygen Consumption. J. Molecul. Cell. Cardiol. 4:139-153, 1972.

9. Harrison, C.E., Jr., Cooper, G., IV, Zujko, K.J., and Coleman, H.N., III: Myocardial and Mitochondrial Function in Potassium Depletion Cardiomyopathy. J. Molecul. Cell. Cardiol. 4:633-649, 1972.

10. Cannon, P.R., Frazier, L.E., and Hughes, R.H.: Sodium as a Toxic Ion in Potassium Deficiency. Metab. 2:297-312, 1953.

11. Edwards, C.: Permeant Ions, Impermeant Ions, Electrogenic Pumps, Cell Volume, and the Resting Membrane Potential. The Physiologist 25:493-495, 1982.

12. Akaike, N.: Development of Pump Electrogenesis in Hypokalaemic Rat Muscle. Pflugers Arch. 379:215-218, 1979.

13. Bilbrey, G.L., Herbin, L., Carter, N.W., and Knochel, J.P.: Skeletal Muscle Resting Membrane Potential in Potassium Deficiency. J.C.I. 52:3011-3018, 1973.

14. Smith, T.W., and Wagner, H., Jr.: Effects of (Na^+ + K^+)-ATPase-Specific Antibodies on Enzymatic Activity and Monovalent Cation Transport. J. Membrane Biol. 25:341-360, 1975.

15. Hansen, O.: Interaction of Cardiac Glycosides with (Na^+ + K^+)-Activated ATPase. A Biochemical Link to Digitalis-Induced Inotrophy. Pharmacol. Rev. 36:143-163, 1984.

16. Erdmann, E., Bolte, H.D., and Luderita, B.: The (Na^+ + K^+)-ATPase Activity of Guinea Pig Heart Muscle in Potassium Deficiency. Arch. Biochem. Biophys. 145:121-125, 1971.

17. Cronin, R.E., Nix, K.L., Ferguson, E.R., Southern, P.M., and Henrich, W.L.: Renal Cortex Ion Composition and Na-K-ATPase Activity in Gentamicin Nephrotoxicity. Am. J. Physiol. 242:F477-F483, 1982.

18. Erdmann, E., and Krawietz, W.: Increased Number of Ouabain Binding Sites in Human Erythrocyte Membranes in Chronic Hypokalaemia. Acta Biol. Med. Germ. 36:879-883, 1977.

19. Chutkow, J.G.: Magnesium, Potassium, and Sodium in "Red" and "White" Muscle in the Rat. Proc. Soc. Exper. Biol. Med. 143:430-432, 1973.

20. Sreter, F.A., and Woo, G.: Cell Water, Sodium, and Potassium in Red and White Mammalian Muscles. Am. J. Physiol. 205:1290-1294, 1963.

21. Campion, D.S.: Resting Membrane Potential and Ionic Distribution in Fast- and Slow-Twitch Mammalian Muscle. J.C.I. 54:514-518, 1974.

22. Yonemura, K.: Resting and Action Potentials in Red and White Muscles of the Rat. Jap. J. Physiol. 17:708-719, 1967.

23. Boardman, L., Huett, M., Lamb, J.F., Newton, J.P., and Polson, J.M.: Evidence for the Genetic Control of the Sodium Pump Density in Hela Cells. J. Physiol. 241:771-794, 1974.

24. Chan, P.C., and Sanslone, W.R.: The Influence of a Low-Potassium Diet on Rat-Erythrocyte-Membrane Adenosine Triphosphatase. Arch. Biochem. Biophys. 134:48-52, 1969.

25. Ford, A.R., Aronson, J.K., Grahame-Smith, D.G., and Carver, J.G.: The Acute Changes Seen in Cardiac Glycoside Receptor Sites, ^{86}Rubidium Uptake and Intracellular Sodium Concentrations in the Erythrocytes of Patients During the Early Phases of Digoxin Therapy are not Found during Chronic Therapy: Pharmacological and Therapeutic Implications for Chronic Digoxin Therapy. Br. J. Clin. Pharmac 8:135-142, 1979.

26. Vaughan, G.L., and Cook, J.S.: Regeneration of cation-transport capacity in Hela Cell Membranes after Specific Blockade by Ouabain. Proc. Nat.

Acad. Sci. 69:2627-2631, 1972.

27. Blaustein, M.P.: The Energetics and Kinetics of Sodium-Calcium Exchange in Barnacle Muscles, Squid Axons, and Mammalian Heart: The Role of ATP. In: Electrogenic Transport Fundamental Principles and Physiological Implications. Editors M.P. Blaustein, and M. Lieberman. Raven Press, New York, 129-147, 1984.

28. Fleg, J.L., Gottlieb, S.H., and Lakatta, E.G.: Is Digoxin Really Important in Treatment of Compensated Heart Failure? Am. J. Med. 73:244-250, 1982.

29. Starr, I., and Luchi, R.J.: Blind Study on the Action of Digitoxin on Elderly Women. Am. Heart J. 78:740-751, 1969.

30. Davidson, C., and Gibson, D.: Clinical Significance of Positive Inotropic Action of Digoxin in Patients with Left Ventricular Disease. Br. Heart J. 35:970-976, 1973.

31. McHaffie, D., Purcell, H., Mitchell-Heggs, P., Guz, A.: The Clinical Value of Digoxin in Patients with Hert Failure and Sinus Rhythm. Quart. J. Med., 1978.

32. Dall, J.L.: Maintenance Digoxin in Elderly Patients. Br. Med. J. 2:705-706, 1970.

33. Hull, S.M., and Mackintosh, A.: Discontinuation of Maintenance Digoxin Therapy in General Practice. Lancet, ii, 1054-1055, 1977.

34. Fonrose, H.A., Ahlbaum, N., Bugatch, E., Cohen, M., Genovese, C. and Kelly, J.: The Efficacy of Digitalis Withdrawal in an Institutional Aged Population. J. Am. Ger. Soc. 22:208-211, 1974.

35. Dobbs, S.M., Kenyon, W.I., and Dobbs, R.J.: Maintenance Digoxin after an Episode of Heart Failure: Placebo-Controlled Trial in Out-Patients. Br. Med. J. 1:749-752, 1977.

36. Norgaard, A., Kjeldsen, K., Hansen, O., Clausen, T.: A Simple and Rapid Method for the Determination of the Number of ^{3}H-Ouabain Binding Sites in Biopsies of Skeletal Muscle. Biochem. Biophys. Res. Comm. 111:319-325, 1983.

37. Knochel, J.P., and Schlein, E.M.: On the Mechanism of Rhabdomyolysis in Potassium Depletion. J.C.I. 51:1750-1758, 1972.

38. Williams, J.A., Withrow, C.D., and Woodbury, D.M.: Effects of Ouabain and Diphenylhydantoin on Transmembrane Potentials, Intracellular Electrolytes, and Cell pH of Rat Muscle and Liver In Vivo. J. Physiol. 212:101-115, 1971.

38a. Clausen, T., Hansen, O., Kjeldsen, K., and Norgaard, A.: Effect of Age, Potassium Depletion and Denervation on Specific Displaceable [^{3}H] Ouabain Binding in Rat Skeletal Muscle In Vivo. J. Physiol. 333:367-381, 1982.

39. Brace, R.A., Anderson, D.K., Chen, W.T., Scott, J.B., and Haddy, F.J.: Local Effects of Hypokalemia on Coronary Resistance and Myocardial Contractile Force. Am. J. Physiol. 227:590-597, 1974.

40. Cameron, I.R., and Ward, J.P.T.: Adaptation of the Sodium Pump to Chronic Potassium Lack. J. Physiol. 316:8, 1981.

41. Cameron, I.R., and Hall, R.J.C.: J. Physiol. 251:70-71, 1975.

42. Akaike, N.: Sodium Pump in Skeletal Muscle: Central Nervous System-Induced Suppression by α-Adrenoreceptors. Science 213:1252-1254, 1981.

43. Gullner, H.G., Lake, C.R., Gill, J.R., Jr., and Lakatua, D.J.: Hypokalemia Stimulates Plasma Epinephrine and Norepinephrine in the Rats. Arch. Int. Pharmacodyn. 260:78-82, 1982.

44. Krakoff, L.R.: Potassium Deficiency and Cardiac Catecholamine Metabolism in the Rat. Circ. Res. 30:608-615, 1972.

45. Fukuchi, S., Hanata, M., Takahashi, H., Demura, H., and Goto, K.: The relationship between vascular reactivity and extracellular potassium. Tohoku J. Exp. Med. 85:181, 1965.

46. Biglieri, E.G., and McIlroy, M.B.: Abnormalities of Renal Function and Circulatory Reflexes in Primary Aldosteronism. Circ. 33:78, 1966.

47. Lown, B., Black, H., and Moore, F.D.: Digitalis, Electrolytes and the Surgical Patient. Am. J. Cardiol. 6:309, 1960.

47a. Clausen, T., Kjeldsen, K., and Norgaard, A.: Effects of Denervation on Sodium, Potassium and [^{3}H] Ouabain Binding in Muscles of Normal and Potassium-Depleted Rats. J. Physiol. 345:123-134, 1983.

48. Kahn, J.B., and Acheson, G.H.: Effect of Cardiac Glycosides and Other Lactones and Certain Other Compounds on Cation Transfer in Human Erythrocytes. J. Pharmacol. Exper. Therap. 115:305, 1955.

49 Wilbrandt, W.: The Mechanism of Action of Cardiac Glycosides. S. Briller and H. Conn, Jr. Editors, The Myocardial Cell: Structure Function and its Modification by Cardiac Drugs. Univ. Pennsylvania Press, Philadelphia, 297, 1966.

50. Lown, B., Weller, J.M., Wyatt, N., Hoigne, R., and Merrill, J.P.: Effects of Alteration of Body Potassium on Digitalis Toxicity. J.C.I. 31:684, 1952.

51. Garb, S., and Venturi, V.: The Differential Actions of Potassium on the Therapeutic and Toxic Effects of Ouabain. J. Pharmacol. Exper. Therap. 112:94, 1954.

52. Lown, B., and Levine, H.D.: Atrial Arrhythmias, Digitalis and Potassium, p. 45. New York, 1958. Landsberger Medical Books, Inc.

53. Page, E.: Precipitation of Ventricular Arrhythmias Due to Digitalis by Carbohydrate Administration. Am. J. Med. 19:169, 1955.

54. Francis, D.J., Georoff, M.E., Jackson, B., and Marcus, F.I.: The Effect of Insulin and Glucose on the Myocardial and Skeletal Muscle Uptake of Tritiated Digoxin in Acutely Hypokalemic and Normokalemic Dogs. J. Pharmacol. Exper. Therap. 188:564-574, 1974.

55. Kjeldsen, K., Norgaard, A., and Clausen, T.: Effect of K-Depletion on the Na-K-Pumping Capacity in Skeletal Muscle and the K-Tolerance of Rats. J. Physiol. 333:367, 1982.

56. Schwartz, A.: Is the Cell Membrane Na^+, K^+-ATPase Enzyme System the Pharmacological Receptor for Digitalis? Circ. Res. 39:2-7, 1976.

57. Sweadner, K.J., and Goldin, S.M.: Active Transport of Sodium and Potassium Ions. Basic Sci. Clin. 302:777-783, 1980.

58. Hazlewood, C.F., and Nichols, B.L.: Changes in Muscle Sodium, Potassium, Chloride, Water and Voltage During Maturation in the Rat: An Experimental Theoretical Study. John Hopkins Med. J. 125:119-133, 1969.

59. Kaplay, S.S.: Erythrocyte Membrane Na^+ and K^+ Activated Adenosine Triphosphatase in Protein-Calorie Malnutrition. Am. J. Clin. Nutr. 31:579-584, 1978.

60. Kim, D., and Smith, T.W.: Effects of Thyroid Hormone on Sodium Pump Sites, Sodium Content, and Contractile Responses to Cardiac Glycosides in Cultured Chick Ventricular Cells. J.C.I. 74:1481-1488, 1984.

61. Grumbach, L., Howard, J.W., and Merrill, V.I.: Factors Related to Initiation of Ventricular Fibrillation in the Isolated Heart: Effect of Calcium and Potassium. Circ. Res. 2:452-1954.

62. Lee, J.J., Woodbury, W., Kirk, E.S., Grant, B.G., Randolph, G.G., and Scheyer, S.C.: Effect of Calcium on Membrane Potentials of Digitalized Frog Ventricle. Circ. Res. 18:747-1958.

63. Ledwoch, W., Greeff, K., Heinen, E., and Noack, E.: The Influence of Chronic Potassium Deficiency on Energy Production, Calcium Metabolism and Phospholipid Composition of Isolated Heart Mitochondria. J. Molecul. Cell. Cardiol. 11:77-89, 1979.