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NEPHROLOGY FORUM

Primary acquired hypoaldosteronism

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Case presentation

A 57-year-old female, a retired beautician, was referred to Parkland Memorial Hospital for evaluation and treatment of persistent hyperkalemia. She had been in excellent health until approximately 12 years earlier, when she underwent a laminectomy for a herniated nucleus pulposus. The surgery was uneventful and her convalescence was uncomplicated except for the loss of 70 lbs (178 to 108 lbs) during the 7-month period that she was confined to a body cast. When the cast was removed, she experienced a syncopal episode. A complete evaluation at another hospital suggested that the syncope was the result of orthostatic hypotension. Pertinent aspects of her workup included a normal electromyogram, electrocardiogram, serum protein electrophoresis, and upper gastrointestinal series. Laboratory findings revealed: hematocrit, 42%; total protein, 8.3 g/dl; albumin, 5.3 g/dl; BUN, 9 mg/dl; T_3 resin uptake, 25%; T_4, 4.8 μg/dl; 24-hour urine 17-OH steroids, 12 mg/24 hr (normal <10 mg/24 hr); and 17 ketosteroids, 7 mg/24 hr (normal 5–15 mg/24 hr). Serum electrolytes were reported as normal. She was given 9-α-fluorocortisol, 0.1 mg/day.

The patient continued to complain of dizziness and had several additional hospital admissions over the ensuing 3 years. Her standing blood pressure was 60/30 mm Hg. Neurologic workups continued to be unrevealing. Serum electrolytes remained normal: sodium, 132 mEq/liter; potassium, 4.5 mEq/liter; carbon dioxide, 24 mEq/liter; and BUN, 18 mg/dl. Treatment with elastic stockings, ephedrine, and an antigravity suit were prescribed. The dose of 9-α-fluorocortisol gradually was increased to 0.6 mg/day.

Hyperkalemia (serum potassium, 5.7 mEq/liter) was documented for the first time 8 years ago. Six years ago the 24-hour urinary aldosterone level was "non-detectable" while she was ingesting a low-sodium diet; serum aldosterone was 2 ng/dl 4 hours after assuming the upright posture and receiving a diuretic, and plasma cortisol after ACTH injection was 2 μg/dl at 4 PM. She also had a subnormal response of plasma cortisol to an 8-hour ACTH infusion but the metyrapone test was normal; 11-DOC increased to 3 μg/dl and serum cortisol fell slightly. She was advised at that time to increase 9-α-fluorocortisol to 1.0 mg/day.

For the past 5 years she has not only been hypotensive, but also severely hyperkalemic, the serum potassium concentrations frequently being greater than 7.0 mEq/liter with a peak value of 9.9 mEq/liter. When the serum potassium exceeded 9.0 mEq/liter, generalized weakness and occasional frank paralysis occurred. Serum potassium values have remained markedly elevated despite therapy with sodium polystyrene sulfonate (Kayexalate). The course has been further complicated by occasional periods of hypokalemia, occurring when the cation exchange resin was increased in conjunction with a decreased intake of potassium. Creatinine clearance 5 years ago was 55 ml/min.

A repeated endocrine evaluation 4 years ago revealed: supine plasma aldosterone, 3 ng/dl (normal 3–10 ng/dl); upright plasma aldosterone, 2 ng/dl (normal 5–30 ng/dl); 24-hr urinary aldosterone, 3 μg/24 hr (normal 4–20 μg/24 hr); supine plasma renin, 3.5 ng of angiotensin generated/ml/hr (normal 0.5–1.6 ng/ml/hr); 2-hour upright plasma renin, 18.4 ng of angiotensin generated/ml/hr (normal 1.9–3.6 ng/ml/hr); 24-hour urinary 17-ketosteroids, 4.8 mg/24 hr (normal 5–15 mg/24 hr); 24-hour urinary 17-OH-steroids, 10.6 mg/24 hr (normal 4–8 mg/24 hr); and plasma cortisol, 30.7 μg/dl at 8 AM and 20.4 μg/dl at noon (normal 7–27 μg/dl at 9 AM). Although a value for serum potassium was not recorded simultaneously, the most likely diagnosis appeared to be isolated hyperreninemic hypoaldosteronism.

One year ago she again experienced periods of weakness. She also had reductions in serum sodium to 106 mEq/liter and elevations in serum potassium to 7.5 mEq/liter despite high doses of sodium polystyrene sulfonate and 9-α-fluorocortisol. However, her symptoms persisted and the metabolic abnormalities remained difficult to control. For these reasons she was admitted to the Clinical Research Center.

Physical examination revealed a pleasant white female who appeared her stated age. The blood pressure was 80/60 mm Hg supine. In the sitting position, the systolic blood pressure was 60 mm Hg, but the diastolic pressure was unobtainable. Respective pulse rates were 60 and 98/min. The respiratory rate was 18/min. She was afebrile. The remainder of the physical examination was within normal limits.

The laboratory findings were: hematocrit, 38.1%; MCV, 102.2 μ^3; sodium, 123 mEq/liter; potassium, 6.3 mEq/liter; chloride, 104 mEq/liter; carbon dioxide content, 15 mM/liter; calcium, 9.5 mg/dl; phosphorus, 3.8 mg/dl; BUN, 33 mg/dl; and creatinine, 1.7 mg/dl. The arterial blood pH was 7.25. Urinalysis disclosed: pH, 6.5; specific gravity, 1.025; and normal sediment. Serum lipids were normal. Serum B_{12} was less than 100 pg/ml (normal 210–920 pg/ml), and folic acid was 3.2 ng/ml (normal 2–14 ng/ml). Electrocardiogram, chest x-ray, and abdominal computerized tomographic scan were normal.

Renal biopsy revealed scattered areas of complete scarring. Other areas appeared completely normal. Specifically the distal tubules and the adjacent interstitium were normal. Each glomerulus, however, displayed a markedly hyperplastic juxtaglomerular apparatus. Electron microscopy and immunofluorescence studies failed to demonstrate any deposits. The biopsy material contained adequate samples both of cortical and medullary sections.

Because of evident salt depletion, the patient was given a diet containing 410 mEq of sodium (9.4 g) and 10 mEq of potassium for the first 2 days. A metabolic diet was then given that contained 260 mEq of sodium (6 g), 10 mEq of potassium, and 3000 ml fluid per day. Successive 3-day collections of stool were analyzed; serum and urine

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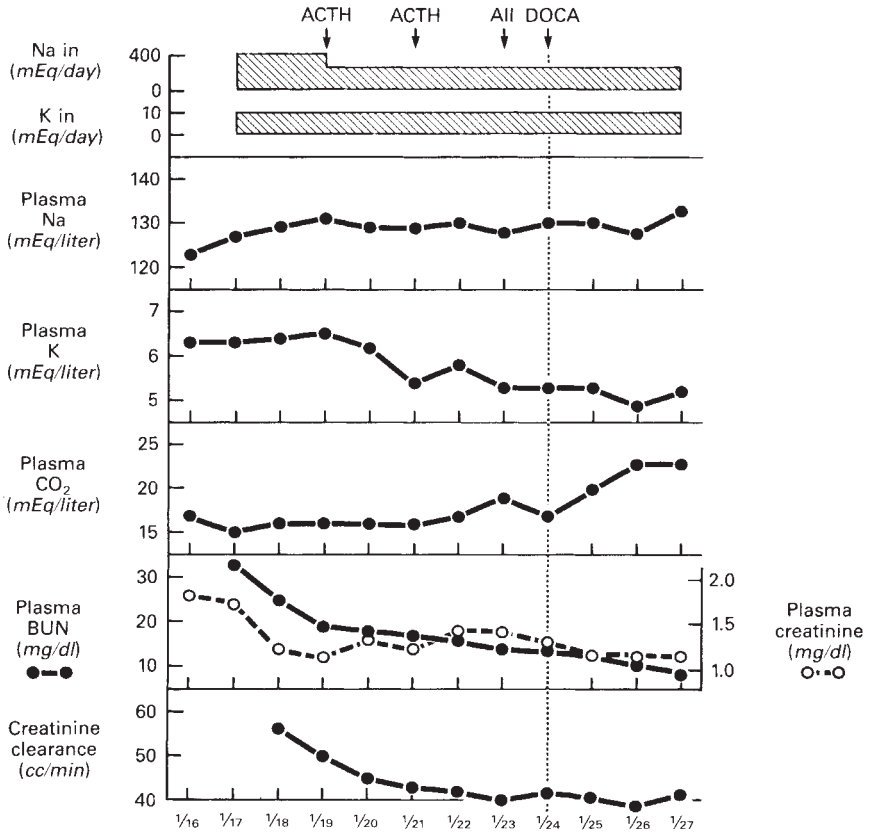

Fig. 1. *Effect of DOCA* (10 mg IM bid) *on plasma sodium, potassium, total CO_2, urea nitrogen, creatinine, and creatinine clearance.*

were analyzed daily. Pertinent metabolic balance data are summarized in Figures 1 and 2. Sodium balance was achieved on the third hospital day.

Renin values were 28.2 ng AI/ml/hr supine, and 43.7 ng AI/ml/hr upright; both values significantly exceed the normal limits. Plasma epinephrine (determined by C. R. Lake at USUHS, Bethesda, Maryland) with the patient in the supine position was 6 pg/ml and 10 pg/ml in the upright position; both values are within the normal range [1]. Plasma norepinephrine was 429 pg/ml (normal 200 ± 9 pg/ml) supine, and 1090 pg/ml (normal 409 ± 29 pg/ml) in the upright position [1]. Values for adrenocorticoids are summarized in Table 1 and Figures 3 and 4; the concentration of aldosterone and its precursor, 18-OHB, were low when interpreted in the light of coexisting hyperkalemia. Furthermore, subnormal increases in these values occurred in response to posture and ACTH infusion, as well as to rates of angiotensin II infusion sufficient to increase blood pressure from 98/68 to 116/82 mm Hg. Baseline glucocorticoid values were normal to high and increased in response to ACTH infusions (see Table 1). These results were interpreted to indicate that the patient had selective unresponsiveness of the zona glomerulosa and a normally responsive zona fasciculata.

Discussion

DR. JUHA P. KOKKO *(Chief, Section of Nephrology, University of Texas Health Science Center, and Professor of Medicine, Southwestern Medical School, Dallas, Texas):* Chronically elevated serum potassium concentration is rare in patients whose glomerular filtration rate exceeds 15 ml/min [2]. When hyperkalemia occurs in association with metabolic acidosis and renal salt wastage, either selective hypoaldosteronism or failure of target organs to respond normally to aldosterone should be considered. Both selective aldosterone deficiency and a failure to respond normally to aldosterone may be the result of inborn biosynthetic errors or acquired defects. Inborn errors in infancy have received the most attention because of their dramatic presentation and more common recognition; however, acquired aldosterone defects in adult patients are being increasingly recognized. The patient under discussion today is unique in that she has a *combination* of acquired selective hypoaldosteronism and a failure of kaliuresis in response to exogenously administered mineralocorticoids, despite the fact that mineralocorticoids given intramuscularly resulted in a typical antinatriuresis and increased hydrogen ion secretion.

Hypoaldosteronism can be the result of a decreased stimulus to the adrenal gland to secrete aldosterone or of an intrinsic adrenal defect in aldosterone secretion. Primary hyporeninemia is thought to be a frequent cause of secondary hypoaldosteronism. Diabetes mellitus and chronic renal insufficiency are most commonly clinically associated with the syndrome of hyporeninemic hypoaldosteronism [2–9]. However, it is not at all clear whether a decreased renin is the cause of the decreased aldosterone secretory rate in all these patients.

A substantial fraction of patients with primary hyporeninemia have an abnormal release of aldosterone in response to intravenous angiotensin II infusion [2]; therefore DeFronzo has suggested that, in addition to hyporeninemia, a primary defect might exist in the adrenal gland. It should be noted, however, that the patient we are discussing today did not have diabetes mellitus and that her renal function was only modestly compromised. Furthermore, her renin values, far from being low, were quite high. Thus she does not fulfill the diagnostic criteria for the hyporeninemic hypoaldosterone syndrome.

The high renin values are consistent with the clinical findings of salt depletion due to renal salt wastage (Fig. 2). The plasma

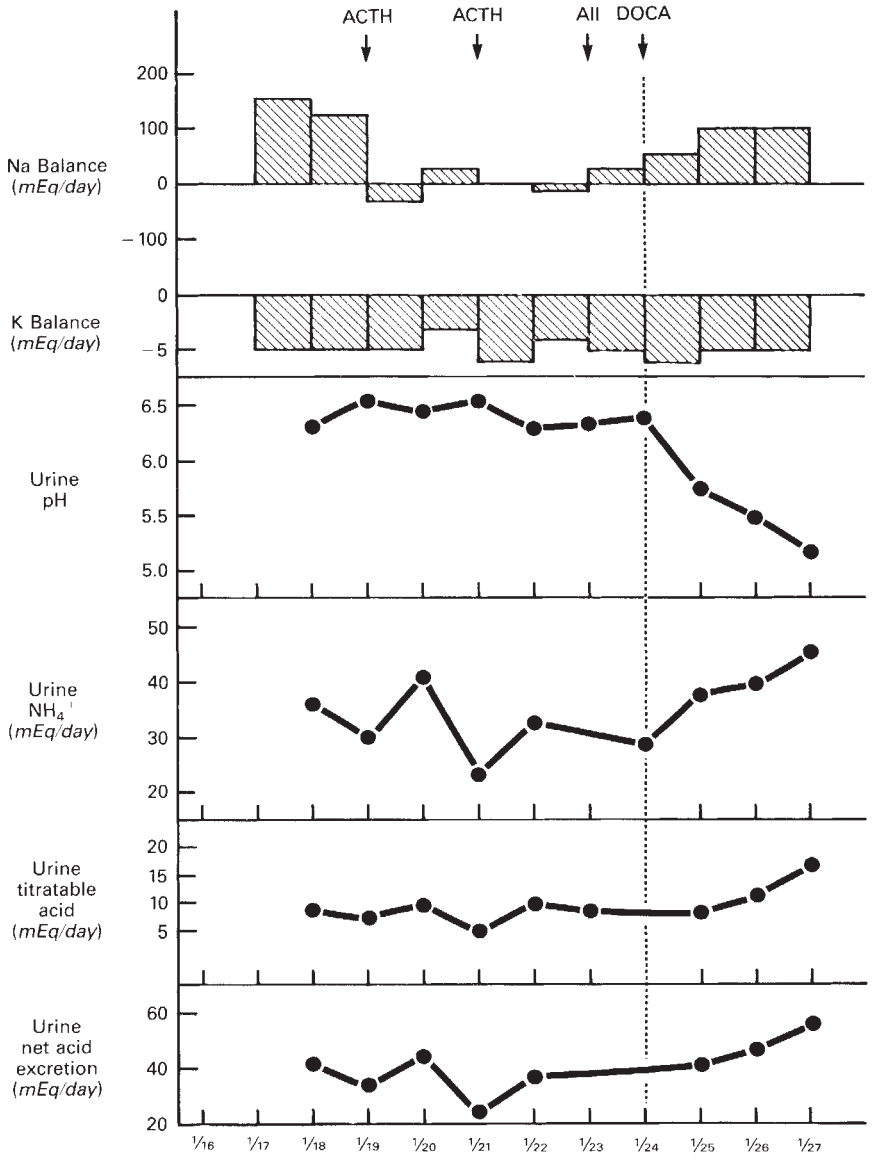

Fig. 2. *Effect of DOCA* (10 mg IM bid) *on sodium balance, potassium, urine pH, urine ammonia, urine titratable acid, and urinary net acid excretion.*

norepinephrine values are also consistent with salt depletion. Previous physicians caring for this patient entertained the diagnosis of idiopathic orthostatic hypotension without diffuse neurologic signs and considered the possibility of the Shy-Drager syndrome. In neurogenic orthostatic hypotension, however, the recumbent plasma norepinephrine values are low and do not rise with upright posture, whereas in the Shy-Drager syndrome, the supine plasma norepinephrine value is normal and does not rise with upright posture [10]. The norepinephrine levels did not rise in any of the patients with neurogenic orthostatic hypotension [10]. Thus this patient's norepinephrine values, which were high in recumbency and rose markedly with standing, are not consistent with a neurogenic origin to her orthostatic hypotension, but rather are most consistent with volume depletion. In further support of this conclusion are the earlier observations that she had little or no benefit from ephedrine administration.

The coexistence of hypoaldosteronism and high renin values raises the possibility that hypoaldosteronism could result from a primary adrenal insensitivity to angiotensin II, due either to a receptor abnormality or to the destruction of the zona glomerulosa. In this patient, we evaluated the aldosterone response to upright posture, to ACTH infusion, and to angiotensin II (see Table 1). Interpreted in the light of coexistent hyperkalemia, the baseline results demonstrate low concentrations of zona glomerulosa steroids (that is, 18-OHB and aldosterone) and elevated concentrations of zona fasciculata steroids (that is, cortisol and corticosterone); DOC and 18-OH DOC can be synthesized in both zones. Furthermore, the results demonstrate a blunted response of the zona glomerulosa steroids to all three of the stimulatory maneuvers employed (see Figs. 3 and 4) [11, 12]. Because the patient had been asymptomatic and had had a normal electrolyte profile until approximately 10 years ago, the present results suggest that she has acquired hypoaldosteronism secondary to dysfunction or destruction of the zona glomerulosa.

Among the more common causes of acquired primary hypoaldosteronism due to a primary defect in the zona glomerulosa are heparin therapy [13, 14], autoimmune disorders [15–19], and

Table 1. Plasma steroid concentrations as influenced by posture, ACTH, and AII infusions[a]

Procedure	Time	Aldosterone *ng/dl*	Cortisol *g/dl*	DOC *ng/dl*	Corticosterone *ng/dl*	18-OHB *ng/dl*	10-OH DOC *ng/dl*
Normal x̄ Values		7.3 ± 2.7	9.9 ± 2.9	7.4 ± 2.8	292 ± 127	22.9 ± 8.2	6.0 ± 3.6
Range		(4–13)	(4–16)	(2–13)	(38–546)	(7–39)	(1–13)
Supine	6 AM	6.8	22.2	41.6	1517	37.7	13.2
Upright	10 AM	8.0	24.8	63.7	2277	39.4	16.4
ACTH infusion							
Control	10 AM	4.6	18.6	15.8	930	20.0	4.5
60′ sample	11 AM	6.9	21.6	44.4	2088	33.4	10.5
AII Infusion (14 ng/kg/min)							
Baseline	3:20 PM	3.6	16.7	17.2	490	16.8	4.5
90′ sample	4:50 PM	4.7	12.8	14.1	814	14.0	3.0

[a] Values were determined by Dr. Morris Schambelan, San Francisco General Hospital. Normal values reflect values determined in his laboratory [9].

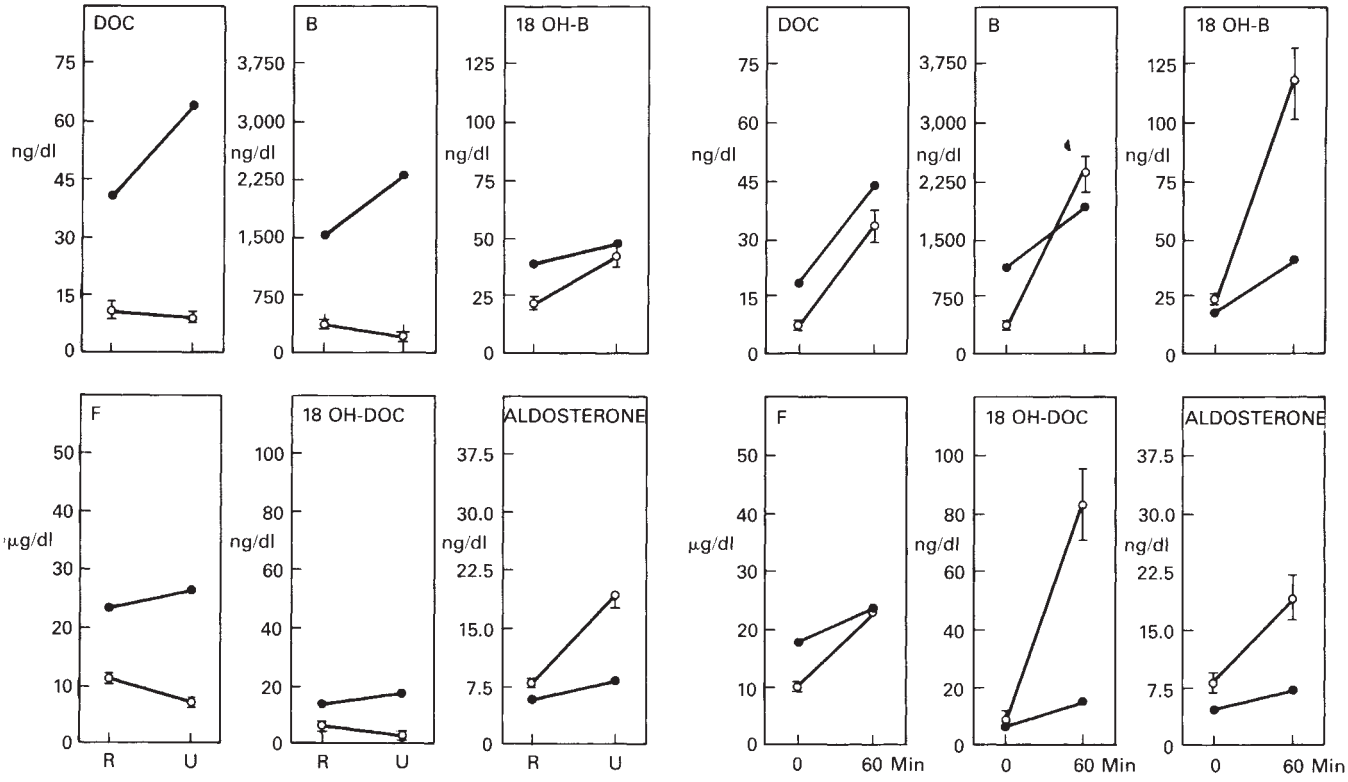

Fig. 3. *The mineralocorticoid response to 4-hour upright posture* (left three panels) *and ACTH infusion test* (right three panels). Patient values are depicted in solid circles; normal values, depicted in open circles, are from Biglieri et al [11].

hypotension in the critically ill patient [20]. The patient we are considering here had not received heparin, and she had not been critically ill with hypotension before she developed the metabolic abnormality. She does have a variety of allergies, but we have not been able to perform tests for autoimmune diseases involving various endocrine organs. Of special interest in this patient would be serum levels of antibodies against the adrenal gland and other endocrine organs. It is possible that she will develop multiple endocrine deficiencies in the future; at present, however, her thyroid and parathyroid hormone levels are normal. It is of interest that her serum B_{12} levels were low, and it is tempting to speculate that this finding also might reflect an underlying autoimmune disorder. Nevertheless, the cause of her zona glomerulosa malfunction remains unknown.

Once the diagnosis of primary acquired hypoaldosteronism was made, it was reasonable to expect a metabolic response to exogenously administered mineralocorticoid. We therefore treated her with intramuscular desoxycorticosterone acetate (DOCA) and elected a high-dose regimen (10 mg intramuscularly twice daily) because of her previous apparent unresponsiveness to oral 9-α-fluorocortisol. The DOCA was initiated on the eighth hospital day. The metabolic responses to DOCA are outlined in Figures 1 and 2. Of interest was the immediate antinatriuretic response, positive sodium balance, and an associated weight gain. The blood pressure returned to 115/70 mm Hg with this therapy; this response constituted presumptive evidence that her hypotension was the result of previous volume depletion due to renal salt wastage. Also, the urine pH,

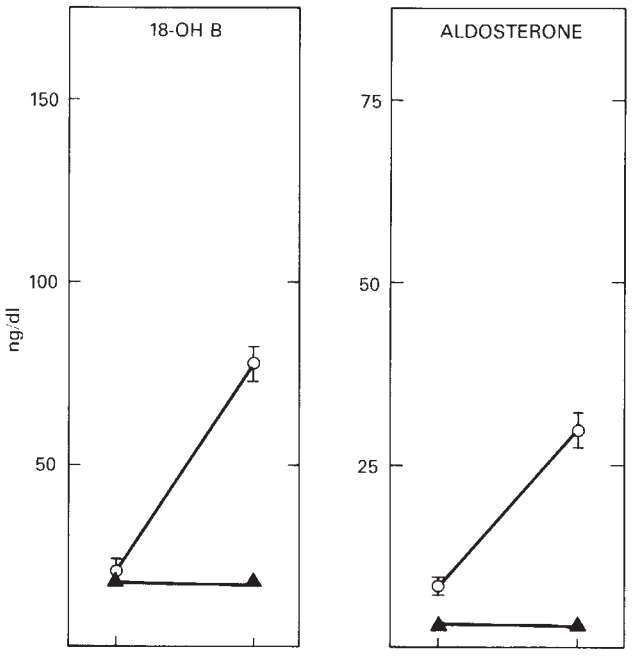

Fig. 4. *Response of 18-OHB and aldosterone to angiotensin II infusion.* Patient values are depicted in solid triangles; normal control values, depicted in open circles, are from Kater and Biglieri [12].

which had been stable at 6.25 to 6.50, immediately began to drop, reaching a level less than 5.50. In conformity, the excretion of ammonium and titratable and net acid increased, and the plasma bicarbonate concentration rose to 23 mEq/liter. However, neither the stool nor urinary potassium rose during the subsequent 4 days of metabolic studies, nor did urinary potassium excretion increase during a month-long period of DOCA therapy. The urinary potassium concentration has remained consistently less than 7 mEq/liter, and the 24-hour urinary potassium excretion has been less than 14 mEq. Thus, the patient appears to have a selective end-organ unresponsiveness to the kaliuretic effects of mineralocorticoids while maintaining a normal response of sodium reabsorption and hydrogen ion secretion.

Failure of kaliuresis in response to aldosterone can be the consequence of a specific defect in hormonal response or can be due to a nonspecific, intrinsic epithelial defect of potassium secretion. To differentiate these possibilities, one liter of 4% sodium sulfate containing 44 mEq/liter sodium bicarbonate was infused. In response to this infusion, the patient had a subnormal kaliuretic response; the highest concentration of urinary potassium following the infusion was 10 mEq/liter, and the greatest rise in fractional excretion of potassium was to 13% from a control of less than 1%. This response, distinctly subnormal when compared to control subjects similarly examined [21], implies that this patient has a collecting duct system that is relatively impermeable to potassium.

The presence of primary hypoaldosteronism in conjunction with an isolated defect in the kaliuretic response to exogenous mineralocorticoids represents a unique set of findings. However, some clinical syndromes do require high doses of mineralocorticoids to correct the metabolic abnormalities. Characteristically, correction of hyperkalemia in diabetic patients with hyporeninemic hypoaldosteronism [2] and in some patients with hyperkalemic varieties of renal tubular acidosis [22] requires high doses of mineralocorticoids. Other circumstances also have been recognized in which normal or elevated values of renin and aldosterone are found but in which the distal tubule fails to respond to aldosterone. Such "pseudohypoaldosteronism" is well described in infants [23] and is being recognized with increasing frequency as an acquired defect in adults. Examples of the latter include renal amyloidosis [24], methicillin interstitial nephritis [25], sickle cell disease [2, 26], and other disease processes affecting primarily the distal tubule [27]. This patient, however, had no evidence on renal biopsy of morphologic abnormalities in the peritubular area of the cortex or medulla. Thus, she does not appear to have any previously described entity, and the reason for her failure to increase potassium excretion at a time when DOCA stimulated both sodium reabsorption and hydrogen ion secretion remains conjectural.

In an effort to shed further light on this patient's problem, I now would like to review our current understanding of how aldosterone affects sodium, potassium, and hydrogen transport at cellular and subcellular levels.

Sodium transport

One of the cardinal features of all forms of hypoaldosteronism is negative sodium balance, principally due to increased renal wastage of sodium. In theory, aldosterone's effect on sodium reabsorption could occur in any nephron segment. In-vitro microperfusion studies have demonstrated, however, that the cortical collecting tubule, not the distal convoluted or medullary collecting tubule, is the principal site at which chronic mineralocorticoid exposure leads to an increase in net sodium reabsorption and an increase in lumen-negative potential [28–34]. Cortical collecting tubules harvested from adrenalectomized rabbits and perfused in the absence of mineralocorticoids have a transepithelial potential close to zero and exhibit negligible levels of sodium reabsorption [28–34]. Thus, there exists a consensus that chronic mineralocorticoid exposure stimulates electrogenic sodium absorption across the cortical collecting tubule.

Figure 5 summarizes the salient features of the cellular and subcellular mechanisms by which aldosterone is now thought to stimulate sodium transport across the cortical collecting tubule. In this model sodium moves down a favorable electrochemical gradient from lumen to cell via conductive pathways ("pores"); this mechanism was demonstrated recently by the direct microelectrode studies of Koeppen, Biagi, and Giebisch [35]. These pathways probably are not completely free diffusion channels, but rather are areas where sodium has some interaction with constituents of the membrane. The permeability of these pathways to sodium is inhibited by amiloride [35]. Regardless of the precise nature of these pathways, sodium diffuses across them from higher (lumen) to lower (intracellular space) energy levels. Intracellular sodium in turn is a sensitive substrate for the sodium-potassium ATPase pump, and the increased availability of sodium results in increased ATPase activity and increased transport of sodium across the basolateral membrane.

Aldosterone could increase transepithelial sodium transport by one of two means, as schematized in Figure 5: an increase in apical membrane conductance to sodium or an increase in

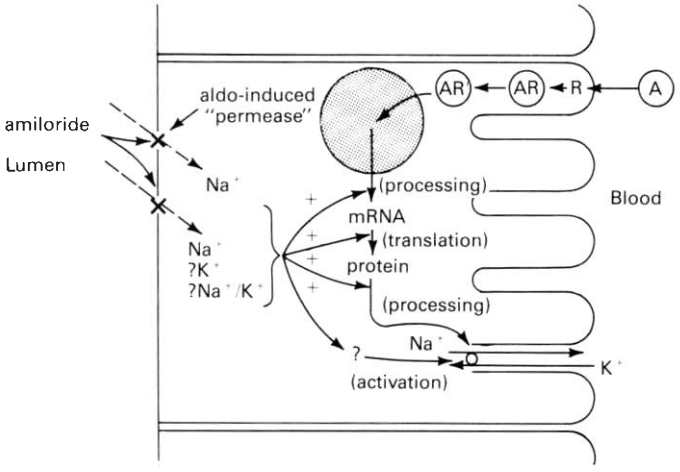

Fig. 5. *Model for secondary enhancement of Na-K ATPase by aldosterone, A.* R is the cytoplasmic mineralocorticoid receptor. In this model, an increase in intracellular Na activity leads to a secondary activation of ATPase, which can be blocked by amiloride [46].

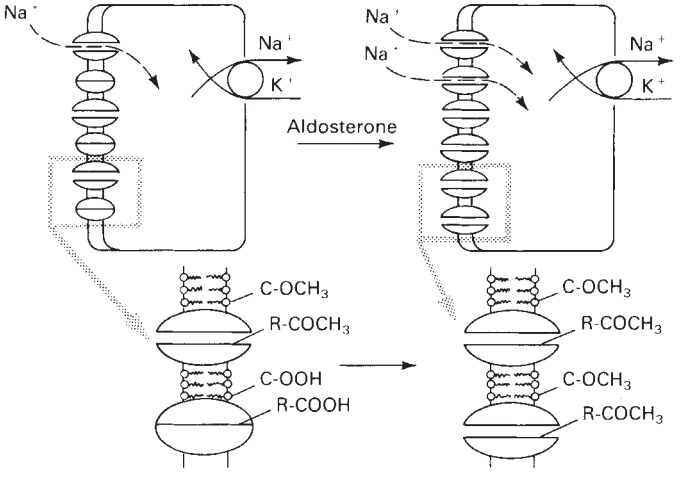

Fig. 6. *Cellular model of the cortical collecting tubule.* Some sodium channels are open and some are closed during control conditions (*left panel*) but all open in response to aldosterone (*right panel*).

sodium-potassium ATPase activity. These two processes could be either independent or mutually dependent. It also is conceivable that one of these processes is rate limiting.

Figure 6 is a highly schematized model that is consistent with much of the available physiologic data regarding aldosterone's influence on transepithelial transport across the cortical collecting tubule. In this model, the rate-limiting step has been placed at the apical membrane. Whereas the evidence for this hypothesis comes largely from studies of frog skin and toad urinary bladder [36–42], the conceptual extension to the mammalian collecting duct is not unreasonable. The sodium channels are depicted in Fig. 6 as being either open (conductive) or closed (nonconductive). As shown on the right side of the figure, aldosterone can be viewed as opening up existing but previously closed channels. The mechanism by which aldosterone might accomplish this task is not known, but exciting preliminary information is evolving. For example, the sodium channels appear to have stereospecificity. Evidence also suggests that the proteins that make up these channels have carboxyl residues [42]. Amiloride is thought somehow to react with these carboxyl groups and close effectively the channel for sodium permeation [42]. It thus is tempting to speculate that sodium conductance through these channels is facilitated by the negative electrostatic forces of the carboxyl groups. How does aldosterone affect the opening and closing of these channels? The answer to this question is not known for certain, but preliminary data from Wiesmann and colleagues suggest that methylation of either the structural phospholipid or permease proteins is involved [43]. This possibility is depicted in the bottom panels of Figure 6. Wiesmann et al added aldosterone to apical membrane vesicles from cultured A6 cells in the presence of methylation inhibitors, and could not demonstrate aldosterone-stimulated sodium transport. The authors therefore suggested that aldosterone somehow stimulates methylation, which in turn increases sodium conductance in the channel. Perhaps the methylation involves the carboxyl groups, as suggested in Fig. 6, but admittedly there are many other ways by which methylation (or other molecular mechanisms) could culminate in an increased number of conductive sodium channels [41]. There is no convincing evidence that, once the sodium gains access to the intracellular compartment, aldosterone in any way regulates its intracellular diffusion (or transport) from the apical to the basolateral membrane; sodium is thought to diffuse freely in the cytoplasmic milieu.

Increasing the activity of the sodium-potassium ATPase pump on the basolateral membrane increases the efflux of sodium from the cell and, in the absence of other epithelial effects, increases transepithelial movement of sodium. Indeed, many studies have shown that treating animals with mineralocorticoids increases the sodium-potassium ATPase activity of the cortical collecting tubule [44–50]. There are three principal substrates for the sodium-potassium ATPase: ATP, potassium, and sodium. In theory, aldosterone could affect any one of these substrates either directly or indirectly. Whereas a large body of evidence supports the view that aldosterone can increase mitochrondrial metabolism and thereby increase ATP availability to the sodium-potassium ATPase pump, mitochondrial performance is not thought to be rate limiting; therefore aldosterone probably does not regulate sodium-potassium ATPase activity primarily by influencing cellular metabolism [51]. Also, the possibility that aldosterone affects sodium-potassium ATPase activity by altering extracellular potassium concentration at the basolateral membrane is unlikely. But, as discussed previously, aldosterone does cause increased entry of sodium from the lumen via the conductive pathways. Aldosterone thus could increase pump activity by increasing the availability of one of the sodium-potassium ATPase substrates, namely sodium. The studies by Petty, Kokko, and Marver have addressed this issue directly [46]. Cortical collecting tubules of adrenalectomized rabbits were shown to increase the sodium-potassium ATPase activity in response to the acute administration of aldosterone only if sodium was permitted to enter the collecting tubule cell. If the rabbits were pretreated with the sodium channel blocker amiloride, the expected rise in sodium-potassium ATPase did not occur. These studies are consistent with the hypothesis that aldosterone increases sodium-potassium

ATPase activity as a consequence of increased apical entry of sodium. In addition, aldosterone might directly affect the synthesis of subunits of ATPase [52], but these effects might not be rate limiting.

Potassium transport

Hyperkalemia is a common finding in untreated patients with severe hypoaldosteronism. Indeed, persistent hyperkalemia in the absence of chronic renal failure, acid-base disturbances, and specific drugs affecting potassium secretion should prompt the physician to consider the diagnosis of hypoaldosteronism.

Whether mineralocorticoids directly affect potassium secretion has been difficult to establish. After an exhaustive review of the literature, Rabinowitz concluded that most reported clearance studies failed to demonstrate an acute effect of aldosterone on potassium secretion [53]. In keeping with this conclusion, most patients given mineralocorticoids do not increase potassium excretion unless they have an adequate intake of sodium [54, 55].

Urinary potassium excretion is primarily regulated by transport processes occurring across distal tubular segments. The transport of potassium across these segments occurs by active as well as passive mechanisms and is influenced by a number of factors, some of which have a direct epithelial effect on the regulation of potassium excretion, whereas others affect potassium excretion secondarily. Factors affecting distal potassium excretion can be divided into two subgroups, tubular (for example, rate of luminal flow, rate of transepithelial sodium reabsorption, and transepithelial potential difference) and systemic (for example, dietary potassium intake, acid-base status, hormonal levels). In view of these complexly interacting factors, experiments designed to determine whether mineralocorticoids have a direct epithelial effect on potassium transport must be conducted under well-defined circumstances in which all the potential influences on potassium transport are controlled. Studies using the isolated tubule technique are interesting in this respect. Recent in-vitro studies have demonstrated that collecting tubules harvested from adrenalectomized rabbits do not increase potassium secretion over the first 120 minutes of exposure to aldosterone in vitro despite a clear increase in net sodium reabsorption [56]. These studies support the view that mineralocorticoids do not directly stimulate potassium secretion acutely in the cortical collecting tubule. However, tubules dissected from rabbits given DOCA chronically have a higher rate of potassium secretion than do tubules dissected from rabbits that have not received DOCA [57–59]. There seems to be a good correlation between plasma levels of mineralocorticoids and the potassium secretory rate of the cortical collecting tubule [58]. The view thus is developing that the cortical collecting tubule responds to aldosterone in a biphasic manner: acutely, there seems to be no direct effect of aldosterone on potassium secretion, but chronically potassium secretion is stimulated [56].

The mechanism of transcellular potassium transport in the cortical collecting tubule and the possible effect of mineralocorticoids on this mechanism have been explored recently with microelectrode measurements. These measurements have disclosed that the mean intracellular potential difference is -84 mV with respect to the blood side [35] and that this tubular segment is composed of two subpopulations of cells with the predominant cell type having a more negative potential difference across the basolateral membrane [60]. With chronic mineralocorticoid treatment, the mean potential difference became more negative in randomly punctured cells (−105 mV) [35]. Thus, chronic mineralocorticoid treatment can increase the influx of potassium across the basolateral membrane by two mechanisms: *(1)* an increase in sodium-potassium ATPase activity as previously discussed, and *(2)* an increase in the passive entry of potassium across the basolateral membrane down a presumably favorable electrochemical gradient [35]. When potassium gains entrance to the cell, it can leave either across the apical or basolateral membrane. Microelectrode studies have demonstrated separate channels for sodium and potassium conductance at the apical membrane with the conductance for potassium being much greater [35, 61]. There also exists a conductive pathway for potassium at the basolateral membrane [35, 60, 61]. But because the lumen of the cortical collecting duct is negative with respect to the blood side under most circumstances, and because the lumen becomes more negative with chronic mineralocorticoid therapy, there is a preferential driving force for potassium to leave the cell across the apical rather than the basolateral membrane. Furthermore, the studies by Koeppen et al have suggested that chronic DOCA administration preferentially increases the potassium conductance of the apical membrane to some extent [35]. These studies indicate that chronic exposure to mineralocorticoids can increase potassium secretion across the cortical collecting tubule cells by mechanisms other than simply increasing sodium-potassium ATPase activity.

In addition to increasing the transcellular secretory movement of potassium, mineralocorticoids also facilitate the paracellular influx of potassium from blood to lumen. The evidence for the existence of this pathway comes from the observations that the cortical collecting tubule is finitely permeable to potassium, and that potassium transport depends on the transepithelial potential difference in a linear fashion [59, 62]. It is not clear what fraction of the kaliuresis induced by aldosterone in vivo stems from transcellular and what fraction from paracellular secretion.

Hydrogen transport

Metabolic acidosis is a common manifestation of hypoaldosteronism. This finding is in keeping with the inference drawn from clearance studies that mineralocorticoids play a role in regulating hydrogen ion excretion [63]. Recent in-vitro microperfusion studies have localized the nephron site of bicarbonate reabsorption and/or secretion, and have explored the role of mineralocorticoids in these transport processes [64–68].

The cortical collecting duct is unique among nephron segments because it can secrete as well as reabsorb bicarbonate. McKinney and Burg, the first to demonstrate this phenomenon, showed that tubules harvested from acidotic rabbits reabsorbed bicarbonate, whereas tubules harvested from alkalotic rabbits secreted bicarbonate [64–66]. Similar results were later obtained by Lombard, Kokko, and Jacobson [67]. Koeppen and Helman further demonstrated that the more negative the lumen potential difference, the more acidic the lumen pH [68]. This finding is not surprising in view of the finite permeability of the tubule to hydrogen ion (1.3×10^{-3} cm/sec) [69]; morever, the finding at least partially explains the reduction in luminal pH

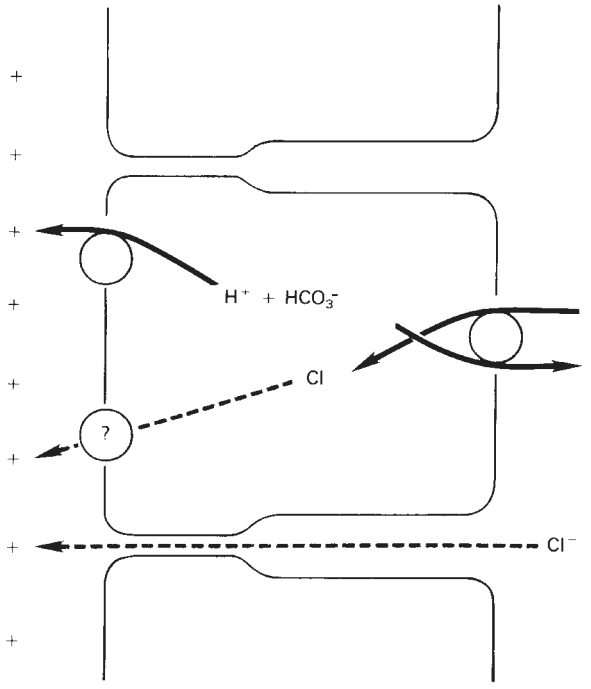

Fig. 7. *Model of hydrogen secretion by the medullary collecting tubule.* Lumen-positive potential is represented by plus signs.

produced by chronic DOCA administration [68]. One might postulate from these observations that the cortical collecting tubule has an important role in the overall regulation of acid-base balance; this may not be the case, however, because the capacity of the cortical collecting tubule to either reabsorb or secrete bicarbonate is limited, being only a fraction of the capacity of the outer medullary collecting duct to reabsorb bicarbonate [67].

In-vitro microperfusion studies have shown that the outer medullary collecting duct always reabsorbs bicarbonate whether the tubules were harvested from acidotic or alkalotic rabbits [67]. This finding stands in marked contrast to the previously mentioned findings in the cortical collecting tubules, where the direction of bicarbonate transport critically depends on the acid-base status of the rabbit.

Figure 7 depicts our current concepts about the transcellular transport of hydrogen ion across the outer medullary collecting duct. According to this view, the transepithelial reabsorption of bicarbonate (or secretion of hydrogen) does not depend on sodium transport. Evidence for this belief stems from the finding that bicarbonate reabsorption is unaffected either by the presence of amiloride in the lumen or by the total replacement of luminal sodium with tetramethylammonium [70]. The sodium-independent, hydrogen ion secretory mechanism is associated with a lumen-positive transepithelial potential difference [67, 70–72] (Fig. 7). To examine the mechanism whereby base exits the cell across the basolateral membrane, we bathed tubules in commonly employed, chloride-containing solutions and in solutions in which chloride had been substituted with gluconate [71]. Chloride removal reduced transepithelial reabsorption of bicarbonate to zero; this finding is most consistent with the presence of a chloride-bicarbonate (or hydroxyl) exchange mechanism at the basolateral membrane. Figure 7 depicts chloride as being secreted along both transcellular and paracellular routes. Although our studies indicate that hydrogen and chloride are secreted at the same rate [71], it is not possible currently to determine precisely what fraction of chloride transport occurs through the cell, because the apical and basolateral conductances to chloride across the outer medullary collecting duct cells have not yet been determined.

Recent preliminary studies by Stone et al, using luminal fraction-enriched vesicles from bovine renal medulla, have provided evidence for the existence of a proton-translocating ATPase [72]. If further studies unequivocally establish the presence of hydrogen-ATPase on the apical membrane, the hydrogen secretory model depicted in Figure 7 will gain further support.

Do mineralocorticoids have a role in stimulating hydrogen secretion? Stone et al have shown that both acute exposure of tubules to aldosterone and chronic administration of mineralocorticoids to rabbits significantly increase bicarbonate reabsorption [70]. This mineralocorticoid-stimulated increase in hydrogen ion secretion occurred in the absence of luminal sodium [70]. Glucocorticoids did not influence bicarbonate reabsorption across the outer medullary collecting duct. In view of these findings and because of the demonstration that aldosterone does not affect sodium transport across the medullary collecting duct [31], I find it attractive to postulate that aldosterone directly stimulates the sodium-independent, proton-translocating ATPase at the apical membrane. Direct studies supporting this conclusion are not yet available, but the possibility remains a fertile topic for future research.

Summary

Of particular interest in the patient under discussion today was an apparently normal antinatriuretic and hydrogen-secretory response to aldosterone, but a virtually absent potassium-secretory response (see Figs. 1 and 2). Indeed, the fact that stool potassium also failed to increase in response to intramuscular DOCA administration suggests that the defect acquired by this patient might not have been limited only to the kidney. The pathogenesis of these findings cannot be adequately explained by the currently available information that I have reviewed. Because the urinary potassium excretion did not respond normally either to mineralocorticoids or to sodium sulfate infusions, I suspect that the apical membrane and the paracellular spaces of the cortical collecting duct of this patient were relatively impermeable to potassium. Unfortunately, this possibility cannot be tested directly.

Questions and answers

DR. ALAN KANTER *(Attending Nephrologist, Michael Reese Hospital):* Have similar studies to those performed on your patient been conducted on individuals with normal renal function who have been treated with pharmacologic doses of 9-α-fluorocortisol, for example, patients with the Shy-Drager syndrome? Second, how long before the results were obtained was fludrocortisone discontinued in this patient?

DR. KOKKO: Yes, the renal effects of 9-α-fluorocortisol have been examined in patients with normal renal function. These patients respond to a normal replacement dose of 0.05 to 1 mg/day by sodium retention and kaliuresis. Unfortunately, there is

no easy measure of adequacy of mineralocorticoid replacement. However, if the patient develops excessive sodium retention manifested by edema, hypertension, or congestive heart failure, then the 9-α-fluorocortisol dose is too high. Patients with Shy-Drager syndrome have autonomic insufficiency and therefore behave as if they are volume contracted. These patients often require much higher doses of mineralocorticoids with potassium supplementation to correct hypotension and prevent hypokalemia. Our patient had not received 9-α-fluorocortisol treatment for several days prior to our studies.

Dr. Jordan J. Cohen: How do you explain your patient's failure to respond to supraphysiologic doses of 9-α-fluorocortisol when she had such a clear-cut response to DOCA, as evidenced by the positive sodium balance?

Dr. Kokko: That is an exceedingly interesting question. At the present time I have no satisfactory answer. The only explanation I can offer is that *(1)* she did not take her 9-α-fluorocortisol as prescribed; *(2)* the oral 9-α-fluorocortisol was not absorbed; or *(3)* the 9-α-fluorocortisol was inactivated by some unknown mechanism. The patient is quite reliable and I think she took her medications most of the time. However, it should be noted that the pH of her urine had dropped from 6.5 to 5.5 prior to the sodium sulfate infusion study. This is relevant because she received 1 mg of 9-α-fluorocortisol the night before the study. Thus, I am not convinced that she is unresponsive to oral mineralocorticoids. Indeed, I am unaware of any previous reports suggesting that a patient responds differently to oral versus parenteral mineralocorticoids if the doses are comparable. Further examination of this question would require absorption studies of radiolabeled 9-α-fluorocortisol.

Dr. Marshall Lindheimer *(Renal Section, Mitchell Hospital, Chicago):* The patient had high renin activity yet responded to angiotensin II infusions in an exaggerated manner. Were basal angiotensin II levels measured, and if they too were high, wasn't the response observed paradoxical?

Dr. Kokko: Although we did not measure basal angiotensin II values, basal renin values were significantly above normal. Our results, however, do reveal a normal pressor response to infused angiotensin. I do not think this response can be considered "paradoxical," but it is interesting that she was able to respond to an additional exogenous source of angiotensin.

Dr. David Bushinsky *(Renal Section, Mitchell Hospital):* Given the patient's severe acidosis, one might have expected that her urinary calcium excretion would be quite large and that she would have radiographic abnormalities of her bones. Did you measure calcium excretion or find radiographic evidence of osteopenia?

Dr. Kokko: Her serum calcium level has been consistently low-normal, ranging usually between 8.4 to 8.6 mg/dl. We did not measure urinary calcium excretion, but because I also was concerned about osteopenia, we measured her bone density and found it to be low.

Dr. Cohen: I think one must be careful about interpreting values for urinary net acid excretion and drawing conclusions about the presence or absence of positive hydrogen ion balance when we don't really have a handle on hydrogen ion production rate. Although I think it likely that she was in positive hydrogen ion balance given her high urine pH and relatively low values for net acid excretion, it is difficult to be certain and doubly difficult to be quantitative. Wouldn't you agree?

Dr. Kokko: Yes, I agree with you completely. A number of previous publications have documented that it is difficult to estimate how much net acid is absorbed from dietary sources and how much is produced endogenously. For these reasons we did not even bother to measure the acid ash content of her diet. It is likely, however, that she is in a steady-state, positive hydrogen ion balance in view of her serum acid-base parameters documenting metabolic acidosis. Indeed, positive acid balance may be contributing to the osteopenia.

Dr. Stephen Gluck *(Renal Section, Mitchell Hospital):* I would like to return to the issue of the acid balance in your patient. Net acid excretion apparently increased about 10 mEq/day over a few days and yet the plasma bicarbonate rose from about 16 to 24 mEq/liter. This rise in bicarbonate seems far greater than can be explained by the increase in renal acid excretion. Could this reflect an influence of aldosterone on cell or bone buffering? Has anyone looked for aldosterone receptors in bone?

Dr. Kokko: The rise in serum bicarbonate concentration from 16 to 24 mEq/liter can result from numerous factors. However, I agree that it is reasonable to postulate that extrarenal factors had a role in increasing the serum bicarbonate concentration. Whereas she was studied on a constant metabolic acid ash diet containing fixed quantities of electrolytes, it is conceivable that mineralocorticoids might change the fraction of acid versus alkali absorbed, or it is possible that aldosterone might influence the participation of bone buffering capacity in maintenance of acid-base homeostasis. Glucocorticoid receptors have been identified in the bone [73], but to my knowledge no one has determined whether bone has mineralocorticoid receptors.

Dr. Fredric Coe *(Renal Section, Mitchell Hospital):* Your patient had hypotension that didn't respond to high doses of 9-α-fluorocortisol. The salt loading produced positive salt balance but still the blood pressure didn't increase. It only responded after she got intramuscular DOC or ephedrine. Do you think that the 1.5 kg body weight gain from intramuscular DOC raised her blood pressure? When you did the salt loading, more than 140 mEq of sodium was retained, so there would have been at least a 1 liter increase in extracellular volume. Several days of a high-salt diet by itself should have given the effect of the DOCA if volume expansion alone could correct the hypotension.

Dr. Kokko: When the patient gained 1.5 kg of weight, her diastolic blood pressure increased significantly to the 70 mm Hg range. I feel that this response is primarily a response to increased circulatory volume, and not a direct response to mineralocorticoids. Perhaps the apparent lack of blood pressure response previously to 9-α-fluorocortisone reflects continued negative salt balance, either the result of relatively low sodium intake (lower than the daily urinary output) or the result of her failure to take the prescribed doses of 9-α-fluorocortisol.

Dr. Coe: I also was wondering about the nature of the membrane disorder. You didn't say what you found in the colon, which might participate in a putative systemic disorder of membranes. Was there a potassium secretory or permeability disorder of the colon? Do you think that she has both an immunologic disorder affecting the adrenal gland and a generalized acquired membrane disorder of permeability, or one or the other?

Dr. Kokko: The stool potassium excretion did not rise in

response to DOCA administration. As you know, the stool normally contains approximately 8% to 10% of the amount of potassium ingested. Although some differences of opinion exist, it is generally believed that mineralocorticoids can increase the fecal potassium excretion rate severalfold. Why our patient did not increase her fecal potassium excretion must remain conjectural without additional studies.

DR. GARY TOBACK *(Renal Section, Mitchell Hospital):* Your presentation suggested that aberrant function at the apical surface of collecting tubule cells could underlie the abnormalities observed in this patient. What is the evidence to support a defect at the apical rather than at the basolateral aspect of the cells?

DR. KOKKO: Because our patient underwent 24-hour balance studies, it is an overextension of the existing data to conclude that the failure to excrete normal amounts of potassium was localized to the cortical collecting tubule. To suggest that the failure of kaliuresis in response to mineralocorticoids is the result of decreased apical permeability of the collecting duct is pure speculation. However, this speculation is based on existing physiologic data. A decrease in potassium secretion could result from a number of factors, but since you ask about preferential localization of the secretory defect to the apical rather than the basolateral membrane I therefore will limit my remarks to those two possibilities. In principle, a decrease in potassium secretion could result from either an increase in basolateral potassium permeability or a decrease in apical permeability. An increase in basolateral potassium permeability would increase efflux of potassium from the cell to the blood side. Here it would be recycled into the cell by the sodium-potassium ATPase pump. This transport would set up a futile cycle. On the other hand, a decrease in apical cell membrane permeability would also decrease potassium secretion but would not increase recycling of potassium across the basolateral membrane. Clearly, either of these two mechanisms could be operative. Normally, permeable apical membrane responds to sodium sulfate infusion by increasing potassium secretion down a more favorable electrochemical gradient. We did not demonstrate this in our studies. Thus, our studies indirectly suggest that the apical membrane of our patient's collecting duct was less permeable to potassium than would be expected under normal conditions.

DR. COHEN: You hypothesized that this patient's adrenal problem might have been autoimmune in nature because she had an associated B_{12} deficiency. Is it possible that her renal tubular problem is yet another manifestation of autoimmunity? We know that patients with lupus can develp hyperkalemia because of a defect in renal potassium secretion. As I recall, many but not all such patients have immunoglobulin deposition around their renal tubules, as revealed by immunofluorescence microscopy. Can it be argued that this patient has a lupus-like defect?

DR. KOKKO: Hyperkalemia, acidosis, and a tendency towards salt wastage comprise a constellation of findings common to a number of disease processes. Lupus is an example. Other examples include various immune-mediated processes. It is interesting that she is allergic to many foods including cheeses and various types of drugs. For that reason I suspected that she had interstitial nephritis in response to some allergen. However, she had no monocytic or eosinophilic cuffing of either the distal or collecting duct segments. Indeed, these segments appeared entirely normal both in the cortex and medulla. Also, immunofluorescence studies failed to show any deposits or abnormalities. The serum studies for collagen-vascular diseases also were negative. Therefore I doubt that her disease process is related to lupus.

DR. ARNOLD BERNS *(Attending Nephrologist, Michael Reese Hospital):* Your patient sustained numerous episodes of rather severe hyperkalemia; however, you did not describe serious or life-threatening cardiac effects. Does your hypothesis include an explanation for her apparent cardiac tolerance of severe hyperkalemia?

DR. KOKKO: You are correct, of course, in pointing out that hyperkalemia is associated with asystole. Indeed, it is rare to see patients survive who have potassium concentrations as high as 10 mEq/liter. In our patient, we have no documentation of cardiac arrhythmias unless her syncopal episodes were related to arrhythmia. In many patients with persistent hyperkalemia, potassium adaptation occurs and the heart becomes protected from the toxic effects of hyperkalemia. The details of cardiac adaptation to hyperkalemia are poorly understood. In potassium adaptation there is an increased renal and gastrointestinal excretion of potassium for any given exogenous load, but this is not the mechanism by which the heart is protected.

DR. KAI LAU *(Renal Division, Michael Reese Hospital):* One of the causes for resistance to the kaliuretic effect of DOCA is potassium depletion. On the face of it, this woman seems, if anything, to have had a potassium surfeit. However, she was receiving a very low potassium diet for several days and this raises the possibility that she might have been potassium depleted. Do you have muscle biopsy data or other evidence to exclude this possibility?

DR. KOKKO: No, I did not perform a muscle biopsy. Indeed, the serum potassium concentration does not necessarily reflect total body potassium stores, but I doubt that she was potasium deficient given her persistently low urinary potassium excretion and frank hyperkalemia of years duration.

DR. DANIEL BATLLE *(Renal Division, University of Illinois, Chicago):* In the patient under discussion, selective deficiency of aldosterone, one of the most common causes of hyperkalemia, was carefully documented. What is unusual is the severity of the hyperkalemia despite the fact that renal function was only moderately impaired. Do you think that impaired extrarenal potassium disposal could have contributed to the hyperkalemia in this patient? The acidosis could be easily explained by the inhibiting effects of hyperkalemia and aldosterone deficiency on ammonium excretion. That acidosis improved when plasma potassium was lowered by DOCA administration supports this possibility. What is very interesting, in my view, is the urine pH, which was above 5.5 at a time when she was acidotic. That requires explanation because, as you noted, patients with aldosterone deficiency are consistently able to lower the urine pH well below 5.5 even when they are only slightly acidotic. Hence, your patient may have a tubular defect for hydrogen ion secretion in addition to having aldosterone deficiency, a pattern that we described in some patients with obstructive uropathy and sickle-cell disease [74, 75]. Perhaps additional studies of urinary acidification might have disclosed such a defect. For instance, a defect in hydrogen ion secretion often can be detected by a failure of the urine PCO_2 to increase normally

after bicarbonate loading; this test is a very sensitive marker of distal acidification. It would also be of interest to know the response of urinary pH and acid excretion to sodium sulfate infusion. The combined analysis of the kaliuretic and acidification response to sodium sulfate in patients receiving mineralocorticoids allows one to distinguish between pure selective aldosterone deficiency and voltage-dependent distal renal tubular acidosis [76]. Inability to lower urine pH below 5.5 and failure to increase potassium excretion normally in response to this maneuver would suggest the presence of a defect for hydrogen ion and potassium secretion likely secondary to impaired distal sodium transport (that is, the presence of a voltage-dependent defect). Again, the patient under discussion is "unique" in that she was able to lower urine pH when large doses of DOCA were given. It should be noted, however, that a fall in urine pH below 5.5 was not observed until the third daily dose of DOCA, whereas in our patients only a single dose of mineralocorticoid was given [74]. Nevertheless, the ability of your patient to lower urine pH with DOCA suggests that collecting duct hydrogen ion secretion can be normalized in certain patients in whom large doses of mineralocorticoid are required because of an intrinsic defect in collecting duct hydrogen ion secretion or a "dormant" transport mechanism owing to prolonged aldosterone deficiency.

DR. KOKKO: I will first deal with your questions concerning extrarenal potassium disposal. It is well appreciated that the hyperkalemia of adrenal insufficiency cannot be the result only of decreased urinary excretion of potassium. Thus it would not be surprising at all if part of her hyperkalemia were due to an abnormal transcellular distribution of potassium. Studies have shown that epinephrine, norepinephrine, and aldosterone have roles in regulating the normal extrarenal mechanism of potassium transport. It should be pointed out that plasma epinephrine values were normal. Whether the elevated norepinephrine values had any role in her persistent hyperkalemia is not clear. Recent studies have shown that stimulation of alpha-adrenergic receptors in healthy human volunteers impairs extrarenal uptake of potassium [77]. We also know that in animals, aldosterone is necessary for normal extrarenal potassium disposal [78]. Thus, it is possible that part of this patient's hyperkalemia is the result of deficient extrarenal mechanisms of potassium homeostasis.

Now to your second question. We did not examine this patient's hydrogen secretory capacity in the detail that you suggested. Specifically, we did not measure her urinary PCO_2. We did measure the urinary pH in response to sodium sulfate infusion. However, our studies were designed mainly to examine potassium secretion and therefore we added bicarbonate to our sodium sulfate infusion. Thus, the urinary pH is locked in an alkaline direction and although we lost the aspect of the test that measures hydrogen secretion, we did gain a measure of potassium secretion. She clearly was unable to increase potassium secretion to normal levels in spite of administration of 9-α-fluorocortisol the night before the test and 10 mg of DOCA intramuscularly on the morning of the test.

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