RENAL PHARMACOLOGY, WITH SPECIAL EMPHASIS ON ALDOSTERONE AND ANGIOTENSIN

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INTRODUCTION

Quantitatively, aldosterone affects sodium transport within the kidney only to a minor degree, but its actions are not limited to the kidney. Furthermore, the sodium-conserving effect is not specific for aldosterone, but is observed with other steroids, such as desoxycorticosterone and 9α-fluorocortisol (9αFF), which for pharmacokinetic reasons are more suitable for studying the actions of mineralocorticoids on sodium transport in the intact animal.

When discussing the pharmacology of aldosterone, one must distinguish between acute effects on intrarenal sodium transport, seen after giving a single dose, and results obtained when the hormone is administered repeatedly over prolonged periods. The complex regulation of aldosterone secretion has also to be considered, in which humoral and other factors acting on the endogenous production and release of aldosterone are affected by feedback mechanisms. In addition, counterregulatory mechanisms may modify the response to exogenous aldosterone. Various apparently contradictory findings can be reconciled by the fact that they were obtained under different experimental conditions that cannot be compared directly with one another.

REGULATION OF ALDOSTERONE SECRETION

Aldosterone release from the cells of the zona glomerulosa of the adrenal cortex is stimulated by angiotensin, ACTH, and potassium supply, and by sodium deficiency, but additional humoral factors, so far not identified, may participate in the control of aldosterone secretion. In isolated rat adrenal tissue incubated under standard conditions, aldosterone production is stimulated not only by ACTH, angiotensin II, and potassium ions, but also by serotonin (5-OH-tryptamine), cyclic 3′,5′-AMP, and various cations, such as ammonium, rubidium, or caesium. Recently it was demonstrated that in zona glomerulosa cells taken from potassium-deficient rats the conversion of corticosterone to aldosterone was markedly reduced (1, 2). Potassium intake stimulated 18-hydroxylase activity in the zona glomerulosa cells and, to a lesser degree, also 11β-hydroxylase activity (3). In vitro, angiotensin has only a mild activity, and ACTH must be given in concentrations higher than those necessary to exert an activity on corticosterone secretion in vivo (4). On the other hand, serotonin, which has a marked effect in vitro, has no comparable action in vivo. However,

it stimulates the secretion of cortisol and corticosterone in hypophysectomized dogs (5). An indole derivative, 6-methoxytetrahydroharman, related to serotonin and isolated from the pineal gland and adjacent structures, was claimed to act as adrenoglomerulotropin (6). In later studies, however, it was concluded that the aldosterone-stimulating factor must have another chemical structure (7).

The action of ACTH on aldosterone secretion largely depends on the state of sodium balance. It is most pronounced in sodium deficiency (8–10) and negligible when sodium is retained. In this respect the effect of ACTH on aldosterone secretion is self-limiting and may also be abolished by the sodium retention induced by cortisol or corticosterone, the primary target hormones of ACTH.

The renin-angiotensin system or, more precisely, angiotensin II stimulates the secretion of aldosterone in various species, including man. However, the initial enthusiasm with which the findings on the renal-adrenal interrelationship had been received (11, 12) was damped by observations that cast doubts on the predominant role of angiotensin in the regulation of aldosterone secretion. In intact and hypophysectomized rats, angiotensin had no effect on aldosterone production (13, 14), except in very high doses (15) or during sodium deficiency (16). In man, sodium deficiency induced a much greater increase in plasma aldosterone concentration than was obtained when angiotensin was infused in doses leading to blood levels comparable to those determined during sodium deficiency (17). Bilateral nephrectomy did not affect aldosterone secretion in either sheep (18) or man (19). In anephric patients, plasma aldosterone concentration was found to be correlated with plasma potassium concentration (20), but various other studies suggest that additional factors may be involved in the stimulation of aldosterone production and secretion (21, 22). Little is known of the chemical nature of such factors or of their significance and regulation.

Acute Effects of Aldosterone on Electrolyte Transport

Aldosterone is characterized as the mineralocorticoid hormone of the adrenal cortex and regulates sodium-potassium linked transport not only in the kidney, but also in other secretory glands and various epithelial systems (23, 24). In the kidney the sodium-retaining and kaliuretic effects of aldosterone were best demonstrable in adrenalectomized animals such as rat, dog, or sheep (23, 25, 26). In intact dogs and in man, sodium retention and kaliuresis were shown in balance studies for aldosterone, DCA, and 9αFF (27–30). Similar effects were observed in intact rats after treatment with either DCA (31) or aldosterone (J. Möhring, unpublished observation). However, 9αFF induced a dose-dependent kaliuresis only, whereas sodium balance was unaffected or even negative when rats received high doses (32, 33). Because, under the influence of 9αFF, a change in the diurnal rhythm of urinary electrolyte and water excretion occurred, it was discussed that in acute experiments the effects of steroids on the kidney might depend on the time of day of the study.

Aldosterone also affects urinary acid excretion. In adrenalectomized rats, acid excretion was diminished but returned toward normal after aldosterone substitution (34). In man, aldosterone or DCA enhanced net acid excretion (35, 36).

Aldosterone was found to exert its action on sodium-potassium transport in almost all vertebrate species that had been investigated, and only recently a certain refractoriness to the renal effects of aldosterone was described for the golden hamster (37). In adrenalectomized hamsters, aldosterone, over a wide dosage range, failed to produce typical sodium retention, but increased potassium excretion. In large doses the mineralocorticoid even had a diuretic and natriuretic effect, probably because of its mild glucocorticoid activity.

Micropuncture studies.—Before studying the effects of aldosterone on sodium transport in various parts of the nephron, it was necessary to define the alterations that occur after adrenalectomy. Besides analysis of tubular fluid obtained at different sites of the nephron in free-flow and microperfusion experiments, measurements of transit time determined by lissamine green, and of fluid reabsorption determined by the split oil-drop method, were done in proximal and distal parts of the nephron. In rats, one week after removal of the adrenals and without dietary sodium supplementation, TF/P[Na^+] and fractional sodium reabsorption in the proximal tubules did not differ from those of controls (38, 39). However, proximal transit time was prolonged, and transtubular sodium reabsorption was diminished. As a consequence of the prolonged transit time, proximal fractional sodium reabsorption was even slightly increased (40, 41). In microperfusion experiments at constant-flow perfusion rates a reduced fractional reabsorption was found (42). Besides the impairment of proximal sodium transport, a decrease in sodium leak permeability was also assumed in adrenalectomized rats (43).

In acute experiments, aldosterone, administered to adrenalectomized rats in doses that corresponded to daily secretion rates, restored the transtubular sodium and fluid transport in the proximal tubules after a delay of about one hour (40, 41). Cortisone had a similar effect, whereas dexamethasone did not normalize the prolonged proximal reabsorption time (44). Actinomycin D, which inhibits the effect of aldosterone on sodium transport in the toad bladder (45–47), also prevented this effect on proximal sodium transport in the kidney (41, 48). Furthermore, in adrenalectomized rats given aldosterone, as well as in intact rats, actinomycin abolished the decrease in proximal TF/P[K^+] observed in free-flow experiments under osmotic diuresis (49). It was concluded that aldosterone modifies net potassium reabsorption in the proximal tubule.

In intact rats an acute effect of aldosterone on proximal tubules is difficult to demonstrate. When the endogenous secretion of aldosterone was suppressed by supplying 1% saline as drinking fluid for four weeks, fractional sodium reabsorption in the proximal tubules was reduced and sodium excretion rose (50). Aldosterone, when given at that stage of the experiment, normalized the intrinsic reabsorptive capacity for sodium (50).

In 20-day-old rats, in which regulation of aldosterone secretion is not yet fully developed and proximal fluid reabsorption time is prolonged, aldosterone enhanced proximal sodium transport to values similar to those found in adult rats (51).

Only few data are available on micropuncture studies in adrenalectomized

dogs treated with mineralocorticoids. Administration of 9αFF reduced sodium and increased potassium excretion in dogs maintained on a high sodium intake and dexamethasone, but proximal fractional sodium reabsorption was either unchanged or slightly decreased (30, 52). In contrast to the rat, proximal transit time and absolute sodium reabsorption were not altered (52). It was concluded that, in dogs, mineralocorticoids have no effect on sodium transport in proximal tubules; however, it must be considered that the experimental conditions differed from those used in rats. Despite the contradictory findings it may be concluded that in some situations aldosterone influences fractional sodium reabsorption in the proximal tubules. However, the significance of this effect for the regulation of sodium balance remains to be demonstrated.

After adrenalectomy, free-flow micropuncture experiments in rats revealed an elevated TF/P[Na^+] at the beginning of the distal tubules (38). It was inferred that sodium transport is impaired in the ascending limb of Henle's loop. After administration of aldosterone or of cortisol, early distal tubular fluid, TF/P[Na^+], and TF/P osm. tended to fall towards normal (53). This effect was more pronounced when aldosterone and cortisol were given simultaneously.

In adrenalectomized rats, TF/P[Na^+] was elevated in the distal tubules as a consequence of a reduced ability to build up a concentration gradient for sodium (38). Fractional sodium reabsorption was decreased and transit time was prolonged (38, 39, 43). In addition, the increase in TF/P[K^+] observed in the distal tubules of control rats was lacking in adrenalectomized animals. Aldosterone and cortisone normalized the elevated TF/P[Na^+] within 45–60 min in free-flow and stationary microperfusion experiments (38), whereas dexamethasone decreased only distal water permeability; this resulted in a hypertonic distal sodium reabsorption (44).

Actinomycin diminished the transtubular sodium transport in the distal tubules of intact rats (41), whereas it had no such effect in adrenalectomized rats. When actinomycin and aldosterone were given together, the normalization of distal tubular sodium transport and of transit time were abolished (41). On the other hand it is assumed that actinomycin does not influence the action of aldosterone on distal potassium secretion. The effects of luminal potassium concentration on distal transepithelial potential differences and on potassium "transport numbers" were studied, by means of the micropuncture technique, in normal and in adrenalectomized rats, the latter with or without aldosterone supplement. The results suggested that aldosterone facilitated potassium secretion mainly by increasing the permeability of the luminal cell membrane for potassium (49). Actinomycin did not suppress this effect (49). These and other findings on the effect of actinomycin on sodium and potassium excretion in urine (26, 49, 54) hint at the possibility that the effect of aldosterone on potassium excretion might be dissociated from that on sodium excretion. Such an assumption is favored by observations in adrenalectomized rats kept on a diet of relatively high potassium content, in which aldosterone caused sodium retention but no kaliuresis (26, 49). When a low-potassium diet was given, aldosterone increased potassium excretion, but it had no effect on potassium excretion when a potassium-deficient diet was

administered (49). In hypophysectomized rats, actinomycin D had no consistent effects on either sodium or potassium excretion, with or without aldosterone supplements (55). A shortcoming of all these studies is that neither sodium nor potassium balances were measured. Furthermore, additional factors, such as distal tubular flow rate, distal sodium load, or changes in hydrogen and bicarbonate excretion, should be considered when the experimental data are interpreted (56, 57).

In functionally isolated collecting ducts of rats, aldosterone increased net sodium transport and reduced leak permeability for sodium (58). In further studies a decrease in ^{24}Na efflux from the collecting ducts was found (59), indicating a reduced sodium permeability of the duct epithelia under the influence of the hormone. This decrease in sodium leak permeability contributes to the increase in the transcellular sodium concentration gradient measured in the collecting ducts. In this part of the nephron the effect of aldosterone on potassium reabsorption or secretion has so far not been determined directly. However, on the basis of micropuncture studies in adrenalectomized rats (39) and in rats on a low-sodium diet (60) it may be assumed that aldosterone favors potassium secretion also in the collecting ducts.

Recently it has been suggested that the effect of aldosterone on the distal parts of the nephron might contribute to "potassium adaptation". After a period of high-potassium intake, rats survived an acute potassium load that would otherwise have been lethal (60, 61). In such rats, potassium excretion was enhanced after potassium loading, and serum potassium concentration did not increase to the levels seen in control rats (60, 61). After adrenalectomy, potassium adaptation was not demonstrable, but it could be maintained by DCA supplements (61). Potassium adaptation was also observed in rats on a low-sodium diet (60, 61), and micropuncture studies revealed that in rats fed either a high-potassium or low-sodium diet, the development of potassium adaptation is associated with an increased capacity of the distal tubules or collecting ducts to secrete potassium (60). Hence, the effect of aldosterone on renal potassium excretion might contribute to potassium adaptation in the rat.

In summary, the various mineralocorticoids studied affect sodium transport at all sites of the nephron. Besides the unique effect that aldosterone has on tubular sodium transport, it changes the permeability for sodium in proximal and distal tubular cells in a fundamentally different way. Different effects on potassium reabsorption or secretion must also be taken into account. No micropuncture studies have been done so far on the effect of aldosterone on net acid excretion, although it is known that the hormone increases acid excretion in man.

Chronic Effects of Aldosterone

When mineralocorticoids are administered repeatedly, the kidney escapes from the sodium-retaining effect after a certain period. Such a transient effect of DCA on sodium balance was observed in dog (62) and man (63) shortly after the corticoid had become available, but the term "escape phenomenon" was not applied before 1958 (27, 64). All mammalian species studied—dog (62), cat (65), rabbit

(66), rat (67, 68), and man—have been shown to escape from the effects of sodium-retaining corticoids. Variations in the onset of the escape phenomenon depend on the dose of the mineralocorticoid, its pharmacokinetics, and on sodium intake (69–71), the differences being mainly of a quantitative nature. The most extensively investigated mineralocorticoids are DCA and aldosterone; only a few studies have been undertaken with 9αFF. Glucocorticoids, such as corticosterone and cortisone, have also been examined, either after administration or after stimulation of endogenous release (72–74). Besides the escape from the action of exogenous corticoids, the influence of a continuously increased secretion of aldosterone has been investigated, as it occurs in primary aldosteronism as a consequence of either an adenoma of the zona glomerulosa or adrenal hyperplasia (75).

Electrolyte balance studies.—Renal escape from mineralocorticoid action is limited primarily to the retention of sodium. Negative potassium balance continues over a prolonged period, and consequently hypokalemia and a decrease in total exchangeable potassium occur during chronic administration of DCA or aldosterone as well as in most cases of primary aldosteronism. Early studies of aldosterone action in dog and man had already revealed that a sodium-restricted diet prevented potassium loss (70, 76), an observation confirmed in a balance study in man during which aldosterone was continuously administered for several days (77). The negative potassium balance and the resultant hypokalemia seen in the presence of adequate sodium intake are the consequences of renal potassium excretion, since there was no evidence of extrarenal, e.g. fecal, potassium loss or of a redistribution of potassium between the intracellular and extracellular fluid spaces (77).

In pregnant dogs, administration of DCA caused sodium retention, and escape occurred after some days. However, potassium excretion never exceeded control values throughout the experiment (78). In nonpregnant dogs the kaliuresis induced by DCA was abolished by estrogens (79). Hence the absence of kaliuresis during DCA treatment in pregnant dogs was attributed to the increased production of estrogens. The sodium-retaining effect of estrogens and of DCA is additive when the two hormones are administered together; this suggests that sodium reabsorption is affected at different sites of the nephron.

In rabbits, prolonged administration of aldosterone induced fecal loss of potassium, but an initial reduction in its urinary excretion resulted in a positive potassium balance. Since hypokalemia developed despite potassium retention, it was assumed that potassium had moved into the cells (66, 80). After a certain period, renal excretion of potassium rose and, together with the continuous high fecal excretion, led to a negative balance and further lowering of plasma potassium. The initial retention of potassium was accompanied by sodium retention, from which the animals rapidly escaped (66). Aldosterone-induced hypokalemia was accompanied by a fall in plasma magnesium, which in view of the maintained external balance was attributed to a shift of magnesium from body fluids into cells in a way similar to that suggested for potassium (80). In

man an increased fecal potassium loss may occasionally contribute to the hypokalemia seen in primary aldosteronism (81).

In dogs, balance studies with DCA revealed that initial sodium retention was related to sodium intake, but that excess potassium excretion did not correspond quantitatively to the amount of retained sodium (71). Rapid onset of escape was seen in animals with a high sodium intake. Weight gain did not correspond to the amount of retained sodium nor to the expected increase in extracellular fluid volume. It was concluded that, during the phase of retention, either water had been shifted from intra- to extracellular space (71) or retained sodium and chloride had been sequestered, without concomitant fluid retention, in compartments other than extracellular space, such as bone (77). On the other hand, it had been claimed previously that DCA might mobilize sodium from bone tissue, thus leading to an increase in total exchangeable sodium (82).

In recent investigations, Möhring et al (31, 83) suggested that, in rats, the escape from DCA might be biphasic. After initial sodium retention and potassium loss, both sodium and potassium excretion returned to normal within 24 hours. This early normalization of sodium and potassium balance was characterized as "first phase" of the escape phenomenon. Subsequently, sodium balance remained in equilibrium, whereas potassium balance again became negative, since urinary potassium excretion continued to exceed intake for the following days ("second phase" of the escape phenomenon). When the 24-hour balance periods were divided into two 12-hour periods, the escape phenomenon was demonstrable only during the night, the period of the rat's main activity, during which approximately 70% of the total 24-hour amounts of water and electrolytes are excreted. It was concluded that, in the first phase of the escape, sodium reabsorption and potassium secretion are reduced in the distal parts of the nephron, with the consequence of a transient restoration of sodium and potassium balances. In the second phase, proximal sodium reabsorption is diminished, leading to an increased distal sodium load, which results in normal sodium and elevated potassium excretion (31, 83). Balance data in man (27, 84, 85) and micropuncture studies in DCA-escaped dogs (30, 86–88) suggest a similar biphasic pattern of the mineralocorticoid escape phenomenon. During continuous infusion of aldosterone, rats escaped from the sodium-retaining effect within four days (68). Diurnal variations in sodium excretion persisted when high doses of aldosterone were administered and when sodium intake was controlled by a constant infusion of 0.9% saline.

Contrary to either DCA or aldosterone, 9αFF does not induce the typical escape phenomenon in intact rats (D. Haack et al., in preparation). Whereas in dog or man the compound had a characteristic mineralocorticoid effect, from which the kidney escapes (28–30), no positive sodium balance was observed in rats over a dosage range of 9αFF from 0.1 mg to 1.0 mg daily. Potassium excretion rose more than with corresponding doses of DCA. A marked glucocorticoid-like effect was demonstrable, including symptoms such as increased urinary volume and weight loss (D. Haack et al., in preparation).

In some contrast to these data are observations that in rats, 9αFF, when given

together with a low-calcium diet, induced an initial sodium retention of four days' duration and a subsequent escape. During the phase of sodium retention, urinary calcium excretion increased, whereas potassium excretion did not change (67). The limited number of observations, however, does not permit us to draw general conclusions from this observation.

When, after the escape from the effects of mineralocorticoids, sodium balance is re-established, the initially retained sodium is not eliminated (27, 71–75). The resulting increase in total body sodium is also maintained when the mineralocorticoid is given over a prolonged period. Only after cessation of mineralocorticoid administration does sodium excretion transiently exceed intake, and the balance becomes negative for several days, whereas potassium is retained (71, 73, 83). Hence, the balance achieved by the renal escape from the sodium-retaining effect of mineralocorticoids is not correlated with a normalization of extracellular fluid volume, but corresponds to a resetting of the regulatory mechanisms in response to the increased extracellular fluid volume.

Target organs of aldosterone other than the kidney do not escape from its sodium-retaining effect. This has been demonstrated in particular for the gut of rabbit (66), rat (68), and man (27, 89), and for the sweat and salivary glands of man (90, 91). Because of this difference between the kidney and the other tissues it may be assumed that the mechanism underlying renal sodium escape is related either to specific effects of aldosterone on various parts of the nephron or to factors that interfere with the action of aldosterone in the kidney but not in other tissues.

Mechanism of sodium escape.—Since sodium intake determines the onset of the escape phenomenon, an attempt was made to relate the escape mechanism to changes induced by sodium retention. It has been suggested that sodium excretion is controlled by extracellular fluid volume, and that the kidney escapes from the sodium-retaining effect when a critical value of extracellular fluid volume is exceeded (92, 93). It is, however, not at all clear how the kidney receives information on variations in extracellular fluid volume and where such information is sensed. In recent years numerous efforts have been made to find an answer to the principal question: is sodium escape the result of an intrarenal regulatory process, with or without the interference of a natriuretic factor? In other words: is it possible to explain the escape mechanism on the basis of refractoriness of tubular cells to mineralocorticoids, or are the actions of the latter antagonized by intrarenal or extrarenal humoral or physical factors that promote sodium excretion (94)? These considerations are not limited to the escape phenomenon, but generally concern the relation between expansion of extracellular fluid volume and sodium excretion. The simplest explanation would be that the escape phenomenon is nothing but the response of the kidney to an increase in extracellular fluid volume. The situation is, however, more complex, since intravascular and extravascular compartments of the extracellular fluid space may be of different significance for the regulation of sodium excretion. Furthermore, changes in fractional reabsorption and in the reabsorptive capacity of various

parts of the nephron will contribute to the natriuresis following the expansion of extracellular fluid volume by saline infusion or by mineralocorticoids.

Variations in "humoral" or "physical" factors and their effects on sodium reabsorption have recently been extensively examined and reviewed (95–97). It is still not known what significance the various factors studied may have for the underlying mechanism of sodium escape. Furthermore, most of the investigations were concentrated on sodium transport in the proximal tubules. Since expansion of extracellular fluid space might affect sodium reabsorption at various sites of the nephron, the following section deals mainly with the localization of changes in tubular sodium transport subsequent to positive sodium and fluid balance.

In micropuncture experiments, different effects of expansion of extracellular space on proximal and distal tubular sodium reabsorption were observed, depending on whether the expansion was limited to plasma volume or encompassed the entire extracellular fluid volume. When, in dogs, plasma volume was enlarged to a similar degree by infusion either of dextran, albumin, or blood on the one hand or of isotonic saline on the other, the decrease in the fractional reabsorption of sodium in the proximal tubules was similar in both cases. However, urinary sodium excretion rose much more after infusion of saline than after nonelectrolytes (98–100), which indicates that, in the latter case, reduced proximal reabsorption was at least in part compensated at more distal sites of the nephron. This assumption was confirmed when distal tubular transport of sodium was inhibited by chlorothiazide, which produced an enormous natriuresis in dogs in which plasma volume had been expanded previously by albumin or dextran infusions (101, 102). Similar observations were made in rats during high-pressure natriuresis. In normal rats the pressure-induced increase in urinary sodium excretion could be abolished by dextran infusion, but this was not possible in rats pretreated with chlorothiazide (103). Intravascular expansion caused a similar decrease in proximal fractional sodium reabsorption both in chronically salt-loaded and in sodium-deficient rats. The enhanced increase in sodium excretion after salt-loading indicated again that changes in sodium transport in the distal parts of the nephron are of significance for the natriuresis following extracellular fluid expansion. However, other results obtained in rats suggested a predominant role of the proximal tubule. Infusion of Ringer's solution caused a greater decrease in fractional sodium reabsorption in the proximal tubule and a greater natriuresis than the infusion of iso-oncotic solution (104, 105). A decrease in absolute sodium reabsorption was observed only after expansion of intravascular and extracellular volumes. It was concluded that a specific inhibition of proximal reabsorptive capacity is responsible for the enhanced natriuresis that follows the isotonic expansion of extracellular fluid compartments. However, the various observations reported are difficult to compare directly because of the different experimental schedules, which in some instances included an increase in plasma volume by more than 50% (104, 105).

When, in dogs, the degree of expansion of the extracellular space was correlated with the decrease in proximal or distal sodium reabsorption and the resulting natriuresis, no proximal inhibition was observed at the state of moderate expan-

sion, but urinary sodium excretion was enhanced. At a higher sodium load, proximal tubular reabsorption decreased and urinary sodium excretion rose further (106). In rats, however, no such relationship was found (107). Gradual expansion of extracellular fluid volume by isotonic saline loading induced a corresponding decrease in fractional proximal sodium reabsorption, but urinary sodium excretion rose only when the expansion of extracellular fluid volume and the reduction in proximal sodium reabsorption exceeded a certain limit. It may be concluded that, after surpassing the transport capacity of the distal nephron, increments in sodium filtrate delivered from the proximal tubule are reflected by an increase in fractional sodium excretion (107). On the other hand, it had been shown in rats that infusion of hypertonic saline may reduce sodium reabsorption in the distal tubules, without affecting it in the proximal tubules (108–110). In a more recent study it was found that urinary sodium excretion was already increased when 1.5 ml of isotonic saline had been given to rats weighing 200 g (111). Since glomerular filtration rate and fractional sodium reabsorption in the proximal as well as in the distal tubules were not significantly changed, it was assumed that sodium reabsorption might have been diminished in the collecting ducts. Interestingly enough, potassium excretion did not increase during the mild natriuresis as it usually does after severe saline loading. Thus, sodium reabsorption might be affected in different parts of the nephron, depending on the degree of extracellular volume expansion.

The significance of parallel variations in extracellular fluid volume and distal sodium reabsorption for the increase in urinary sodium excretion was also demonstrated in DCA-escaped dogs. At the onset of escape, fractional sodium reabsorption in the proximal tubules had not changed (86–88), which indicated a reduction in sodium reabsorption at more distal sites of the nephron. This assumption was supported by the finding that albumin infusion in DCA-escaped dogs caused a similar reduction of proximal sodium reabsorption as in normal dogs, but a greater urinary excretion of sodium (86). In earlier studies a diminished proximal sodium reabsorption in DCA-escaped dogs had been claimed (30). Since, in these experiments, escape had occurred some days previously, dogs, like rats, may have a biphasic pattern of mineralocorticoid escape (93). In rats, micropuncture studies were only performed after several weeks of DCA-treatment, and at that time fractional sodium reabsorption was diminished in the proximal tubules (112, 113).

In an attempt to differentiate between the significance of plasma volume and that of interstitial fluid volume for the excretion of sodium after saline loading, dogs in various states of sodium and water balance were investigated (101, 102). By mild dehydration of DCA-escaped, salt-loaded dogs or by hydration of sodium-depleted dogs plasma volume—but not the calculated interstitial fluid volume—was normalized. Since dehydration of salt-loaded dogs had no influence on increased sodium excretion and since, in sodium-depleted, hydrated dogs, elimination of a sodium load was still diminished, it was claimed that for the control of tubular sodium transport interstitial fluid volume was more important than plasma volume (101, 102).

When, in DCA-escaped and in control dogs, the aorta was constricted above the origin of the renal arteries, proximal sodium reabsorption markedly increased only in the controls. During reduction of glomerular filtration rate, glomerulotubular balance was maintained in the DCA-treated dogs, but not in the control dogs (8). The prevention of enhanced proximal tubular reabsorption was ascribed to the suppressed activity of the renin-angiotensin system seen in DCA-escaped dogs. However, additional constriction of the aorta stimulated plasma renin activity both in control and in DCA-treated dogs, but the absolute increase in plasma renin activity was lower in DCA-treated dogs than in control dogs. Previously it had been proposed that the renal escape from the sodium-retaining effect of DCA might be related to the depletion of renal renin (114), but the observation that dogs with severe cardiac failure did not escape from DCA despite a suppressed renin activity (115) was brought up against this hypothesis (30). In this case, however, it cannot be excluded that a regulatory mechanism may have been overridden by additional pathophysiological changes, and the possibility that suppression of renin may be of significance for the renal escape from the sodium-retaining effect of mineralocorticoids may still be taken into consideration.

In isolated perfused kidneys from DCA-treated rabbits the response of glomerular filtration rate and filtration fraction to endogenously released and to exogenous angiotensin was diminished (116, 117). This indicates that intrarenal hemodynamics and, consequently, sodium reabsorption may be affected by the activity of the renin-angiotensin system within the kidney.

In summary, there is good evidence that expansion of extracellular fluid volume, either by DCA treatment or by saline loading, affects tubular sodium reabsorption not only in the proximal tubule, but also in distal parts of the nephron. Under conditions as yet not well defined, changes in distal sodium reabsorption may play a predominant role in sodium excretion. Attempts to relate a reduced sodium reabsorption at different sites of the nephron with the degree of extracellular fluid expansion have only partly succeeded so far. That the renin-angiotensin system is of significance for the escape mechanism has not yet been convincingly demonstrated, but not disproved either.

Prevention of sodium escape.—Escape from the sodium-retaining effect of mineralocorticoids was prevented in dogs with experimental heart failure due to an aorta-to-vena cava (A-V) fistula (115). The failure to escape was ascribed to the diminished delivery of sodium to the distal tubules. This assumption was confirmed by micropuncture studies, which also revealed a decreased glomerular filtration rate when compared with DCA-escaped dogs (87). After saline infusion, fractional proximal sodium reabsorption fell in both escaped dogs and non-escaped fistula dogs, but in the latter, sodium excretion rose by only one fourth of that in escaped dogs. It was concluded that an enhanced sodium reabsorption in the distal tubules may contribute to the failure to escape. As a consequence of increased proximal sodium reabsorption, urinary potassium excretion did not rise and hypokalemia, which usually accompanies sodium escape, was not observed (115). However, in dogs with constriction of the thoracic vena cava

inferior, no decrease in proximal sodium reabsorption was demonstrable after saline loading (118).

In summary, besides an increase in proximal sodium reabsorption, changes in distal sodium transport may be essential for the failure to escape.

Mode of Action of Aldosterone

Studies on the mechanism by which aldosterone regulates active sodium transport across cell membranes have made wide use of the isolated toad bladder (*Bufo marinus*) or the urinary bladder and the skin of the frog (*Rana temporaria*), which may serve as models for sodium transport in the distal renal tubules (45, 46, 119–122). Another tissue studied with respect to sodium transport affected by aldosterone is the toad colon (123). In dog experiments it had been noted that the sodium-retaining effect of aldosterone or of 9αFF was demonstrable only after a latent period of 40–60 min, even when the corticoids had been injected directly into the renal artery (23). A similar delay in the onset of action (60–90 min) was observed in the isolated toad bladder (120, 124). Since it was not possible to shorten the latent period by increasing the concentration of aldosterone, Crabbé (124) assumed that the formation or activation of an intermediate might be responsible for the delayed action. At the same time, Williamson (54) and Edelman et al (45) presented data suggestive of aldosterone to induce the *de novo* synthesis of protein(s) by stimulating RNA synthesis. These observations, made in adrenalectomized rats (54) and in the isolated toad-bladder system, encouraged research on the intracellular action of aldosterone, to which Edelman and his associates have made substantial contributions since (125–128).

Evidence for the intracellular site of action of aldosterone was first obtained by high-resolution autoradiographs of toad-bladder epithelium, in which tritiated aldosterone was found to be concentrated over the nuclei of epithelial cells (45, 129, 130). Autoradiographic studies in a number of vertebrates, such as goldfish, salamander, chicken, and mouse, showed that, within 1–8 hours after injection, tritiated aldosterone was concentrated in cell nuclei of various tissues, including those not previously considered to be physiological target organs of the hormone (131). Mineralocorticoids, such as 9αFF, displaced tritiated aldosterone from most of its nuclear binding sites in the toad bladder, whereas estradiol-17β was inactive in this respect (132). By means of quantitative autoradiographic studies mineralocorticoid-binding systems in both the nuclear and cytoplasmatic fractions of toad-bladder epithelial cells were characterized (133). Other investigators observed a positive correlation between the mineralocorticoid activity of various steroids (with the exception of progesterone) and their ability to displace ^{3}H-aldosterone from binding sites in toad-bladder cells (134). In cell fractionation studies about 60% of specific aldosterone-binding sites were localized in the nuclear fraction of toad-bladder cells (135).

Induction of aldosterone-binding proteins (*ABP*).—Extensive studies on the formation and localization of ABP have been done in kidney homogenates from adrenalectomized rats. Among the various cell fractions prepared (nuclei,

mitochondria, microsomes, cytosol), the highest concentration of tritiated aldosterone was found in the nuclear fraction, followed by the cytosol, mitochondria, and microsome fractions (136, 137). The nuclear fraction showed saturation kinetics in the uptake of tritiated aldosterone, which were demonstrated by competition with 9αFF, which, in a 75 times higher concentration, inhibited the uptake of tritiated aldosterone, whereas 6α-methylprednisolone or estradiol-17β was ineffective. In the other cell fractions, 9αFF had no effect on the specific binding of aldosterone to protein. The isomer of 17α-isoaldosterone, which is devoid of mineralocorticoid activity, neither reacted with the nuclear aldosterone-binding sites nor competed with the formation of the aldosterone-protein complex in nuclear fractions (137). On the other hand, a spirolactone derivative (SC 14266), which acts as aldosterone antagonist, inhibited the uptake of tritiated aldosterone by the cytosol and nuclear fractions of ABP (138).

The binding of aldosterone with proteins was also demonstrated by the release of tritiated aldosterone from prelabelled nuclei after incubation with proteolytic enzymes, such as chymotrypsin, trypsin, or pronase. In contrast to broad-spectrum proteases, which caused an accelerated cleavage of the complex, enzymes such as DNAase, RNAase, lipase, or neuraminidase had no effect. Binding activities of the nuclear and cytosol proteins were destroyed by incubation with sulfhydryl reagents, indicating that intact SH groups of cystine or methionine may be responsible for the binding activity (137, 139).

ABP were isolated not only from the kidney, but also from other tissues (140). High nuclear- and cytosol-binding activities were obtained in the duodenal mucosa, whereas the cytosol fractions of spleen, liver, and brain showed only little aldosterone-binding activity. However, the nuclear fraction of the spleen contained approximately 40% of the binding activity of the kidney, and also in the nuclear fractions of liver and brain some binding was observed (139, 140). The significance of nuclear aldosterone binding in tissues in which the hormone has little, if any, effect on sodium transport remains unclear. After injection of tritiated aldosterone into adrenalectomized rats a rise in the acetylation of the arginine-rich histones of the kidney was observed. Similar effects were obtained with 9αFF or DCA, whereas the simultaneous administration of the spirolactone derivative SC 14266 inhibited this action of aldosterone (141).

Actinomycin D, which blocks mRNA-synthesis, inhibited the response to aldosterone in the toad-bladder system (45, 54, 142). Similarly, substances that interfere with protein synthesis at the ribosomal level, such as puromycin or cycloheximide, blocked the mineralocorticoid action in the toad-bladder system (45, 139, 142). On the other hand, compounds that affect DNA-synthesis, such as phleomycin or hydroxyurea, had no effect on the action of aldosterone in the toad-bladder system (143). It was inferred that, for its action, aldosterone requires intact RNA-synthesis, but that there is no such dependence on DNA-synthesis. Cycloheximide, when added several hours after the exposure of the toad-bladder system to aldosterone, had no influence on the mineralocorticoid effect (139). The conclusion was that ABP are accumulated during the induction process, and that inhibition of protein synthesis does not interfere with preformed

ABP. On the other hand, aldosterone itself has no stabilizing effect on ABP (139).

So far, three different ABP have been characterized: the extranuclear cytosol fraction and two intranuclear fractions. Of the intranuclear proteins, one is released by osmotic shock and is soluble in Tris-$CaCl_2$ buffer; the other, which is extracted by KCl, is present in the chromatin fraction (144). The chromatin fraction contained 55% of the tritiated aldosterone taken up by nuclei of kidney cells, the binding being stereo- and mineralocorticoid-specific. The aldosterone-chromatin complex was heat-labile and cleaved by chymotrypsin and trypsin, but resistant to DNAase and RNAase, which indicates that the binding protein is a nonhistone. Of the tritiated aldosterone, 28% was bound to the protein released by osmotic shock, and 17% was combined with the chromatin-free material (144).

The sequence of formation of the three different ABP was studied both in the kidneys of adrenalectomized rats injected with aldosterone and in kidney and parotid slices incubated with tritiated aldosterone (133, 145). Based on the results obtained, a three-step hypothesis was postulated, according to which the cytosol fraction is formed first and its aldosterone complex gives rise to the nuclear complex, which subsequently generates the chromatin complex. The cytosol fraction may be considered the precursor of the intranuclear fractions. In the nuclei, a preliminary conversion step may induce the formation of the Tris-buffer-soluble protein. Subsequently, this protein is responsible for the production of the chromatin fraction, which may function as a receptor system in the aldosterone-induced synthesis of RNA (126). On the other hand, RNA-synthesis in the toad bladder was found to be extremely slow, and the *de novo* synthesis was not accelerated in response to aldosterone (146). Similarly, in nuclear fractions from heart and kidney of adrenalectomized rats no significant effect of aldosterone on RNA synthesis was observed (147).

In rabbits, under the influence of aldosterone, ^{14}C-leucine was incorporated at an elevated rate into protein fractions from renal cortex, an effect prevented by actinomycin D (148). By means of the sequential double-pulse technique, using ^{3}H-leucine and ^{14}C-leucine, it was shown that, during the incubation period, aldosterone stimulated the incorporation of leucine into protein prior to the action on sodium transport. This incorporation was inhibited by the aldosterone antagonist spironolactone.

Besides specific ABP, an unstable and relatively nonspecific protein with aldosterone-binding activity was isolated from the rat kidney (149), but nothing is known of the significance of this protein for the action of aldosterone.

Effects of aldosterone-induced proteins on sodium transport.—Although only indirect evidence is available for a *de novo* protein synthesis under the influence of aldosterone (150), it is generally accepted that the hormone initiates the formation of intermediates responsible for its effect on sodium transport.

The energy-supplying action of ABP is supported by their activating effect on mitochondrial enzyme systems (125, 139, 151). Substrates of oxidative metabolism, such as pyruvate, are necessary for a response of the toad bladder to

aldosterone (45). Inhibitors of NADH oxidation, such as rotenone or amobarbital, abolished the toad-bladder response to aldosterone (152), and it was concluded that ABP stimulate NADH-linked oxidative phosphorylation (125, 139). Increased activity of several mitochondrial enzymes, such as citrate synthetase, isocitrate dehydrogenase, and malate dehydrogenase, was seen in toad-bladder cells exposed to aldosterone (153, 154). In kidney cells, aldosterone also stimulated citrate synthetase, and an increase in ATP was observed (155). The stimulated activity of citrate synthetase was accompanied by the characteristic change of the Na/K ratio in the urine of adrenalectomized rats that had received aldosterone. The effect of adrenalectomy on both Na/K ratio and citrate synthetase could be abolished by aldosterone (155). In recent studies on changes in the redox state (lactate/pyruvate and β-OH butyrate/acetoacetate) of kidneys of rats treated with aldosterone, a redox shift was found for both cytoplasmatic and mitochondrial NAD/NADH ratio (126). These results support the hypothesis that aldosterone, by means of ABP, regulates active sodium transport by supplying energy for the extrusion of sodium from the cells.

Opinions on the site of action are still controversial. Some investigators favor the hypothesis that aldosterone facilitates the entrance of sodium into the cells from the lumen of the tubule (24, 47, 121, 156), while others claim that the extrusion of sodium from the cells into the peritubular space is controlled (126, 139, 157). It is generally accepted that ADH acts on the apical surface of the epithelial cells of the toad bladder (see 158). Since, in this tissue, aldosterone and ADH have additive effects on sodium transport, it was supposed that the two hormones might act at the same site of the cell (47). In isolated (scraped) epithelial cells of the toad bladder, aldosterone and vasopressin stimulated sodium transport, and it was suggested that the two hormones accelerate a rate-limiting step for the entry of sodium into the cells at the mucosal site (159–161). In addition, active sodium transport across the basal membrane of epithelial cells was affected (157, 162). The polyene antibiotic amphotericin B, which disrupts the apical border of toad-bladder cells and facilitates the entry of sodium into the cells, was shown to block the effects of aldosterone on sodium transport (163, 164). In the presence of substrates such as pyruvate or acetoacetate, which are necessary for the action of aldosterone on sodium transport, amphotericin stimulated sodium transport in toad-bladder cells. Since amphotericin does not induce protein synthesis, it was concluded that the effects of pyruvate substrate in the toad bladder are secondary to the entry of sodium into the cells, and that the effects of aldosterone on the substrate are similarly mediated (163). However, doubts have been expressed as to whether amphotericin B is a suitable tool for studying the site of action of aldosterone.

Support for a facilitated apical entry of sodium under the influence of aldosterone has been presented by André and Crabbé (165), who showed that aldosterone potentiated the response of the toad skin to insulin, which may affect the sodium-pump mechanism. In studies with the diuretic amiloride in frog skin or toad bladder and colon it was found that amiloride, like triamterene, inhibited sodium transport by acting on the apical border of the cells

(166–168). Both diuretics were less active when the toad skin was exposed to aldosterone (156).

A two-step response to aldosterone has also been claimed, according to which both the mucosal barrier and the ion pump are affected by aldosterone (162, 169). The major effect of aldosterone on the toad-bladder epithelium is the increase in mucosal sodium permeability, but the hormone may also supply high-energy intermediates in case the sodium pump is rate-limiting. In the urinary bladder of the frog (*Rana temporaria*) an independent stimulation of sodium entry and sodium extrusion was found in the epithelial cells under the influence of aldosterone (122). In addition, a sex dimorphism in sodium content of urinary bladder wall was observed, since aldosterone decreased sodium content in males but increased it in females (170).

Aldosterone may also affect physical factors, and it was claimed that sodium transport across the epithelial cells might be stimulated by a reduction of electrical resistance in the toad bladder (171). This finding supports the theory of a primary action of aldosterone on the apical membrane, since entrance of sodium into the cell would reduce transepithelial electrical resistance. Dependence of sodium transport in the toad bladder on temperature changes was also taken as evidence for an action of the hormone on the luminal side of the cells (172).

The data available do not permit conclusions as to the site of action of aldosterone in the tubular epithelia. There is good evidence for the fact that the hormone favors the apical entrance of sodium into the cells, and that in this way more sodium is made available to the transport system. It can, however, not be excluded that aldosterone also provides energy to the pump mechanism responsible for the extrusion of sodium from the cells to the peritubular capillaries.

Effect of aldosterone on ATPase activity.—The claim that aldosterone may favor sodium extrusion from the cells by supplying energy for the active transport raises the question whether it may also affect sodium-potassium dependent ATPase activity. After adrenalectomy in rats, kidney ATPase activity fell slowly to low levels (173, 174). However, in recent studies, sodium-potassium dependent ATPase activity was found to be increased after adrenalectomy in the plasma membrane fraction prepared from rat kidney homogenates (175). In the microsomal fraction the same authors found a decrease in ATPase activity. When corticosterone was given for substitution, ATPase activity was restored within 2–3 days. In contrast, aldosterone, in physiological doses, had no effect on ATPase activity (173, 174, 176). However, after prolonged administration of aldosterone, a partial restoration of ATPase activity was observed (173). On the basis of these results it was concluded that aldosterone is not involved in the regulation of sodium-potassium dependent ATPase in the kidney. The increase in ATPase activity in the plasma membrane fraction seen after adrenalectomy could be prevented when aldosterone and dexamethasone were given. It was assumed that neither the plasma membrane fraction nor the microsomal ATPase is directly regulated by aldosterone, but that they depend on changes in renal sodium reabsorption (175).

In more recent experiments it was demonstrated that, in adrenalectomized rats, repeated injections of aldosterone increased the activity of sodium-potassium dependent ATPase prepared from the total kidney after 14 and 24 hours, whereas corticosterone was ineffective during this period. However, 24 to 48 hours after adrenalectomy, aldosterone had only a partial effect, whereas glucocorticoids restored the activity to normal levels (176, 177). The most pronounced increase in ATPase activity under the influence of aldosterone was found in the outer renal medulla, while only an insignificant effect was seen in the outer cortex. In the inner cortex, ATPase activity was also increased, but higher doses of aldosterone were necessary than in the outer medulla. Former studies may have failed to demonstrate a clear effect of aldosterone on the ATPase activity, because the periods of observation were too short and did not correspond to the slow rise in enzyme activity, which began only six hours after the administration of the hormone (176, 178). The delayed response of ATPase to aldosterone suggests that it may induce a *de novo* synthesis of the enzyme, an effect that could be interpreted as an adaptation to the sustained increase in sodium reabsorption under the influence of aldosterone (178).

In renal subcellular fractions from intact rats, aldosterone stimulated the Na^+-K^+-dependent ATPase activity, whereas the opposite effect was obtained in similarly prepared subcellular fractions from intact mice. Carbonic anhydrase activity was inversely affected by aldosterone, which enhanced it in mice, but reduced it in rats (179). The aldosterone-induced increase in renal carbonic anhydrase activity was prevented by actinomycin D. On the other hand, aldosterone blocked the inhibitory effect of actinomycin D on the stimulated carbonic anhydrase activity (180). No effect of aldosterone was observed on Na^+-K^+-dependent ATPase activity in the toad bladder, and the aldosterone-induced stimulation of sodium transport in this organ is independent of the enzyme (181).

Role of the Kidney in Aldosterone Metabolism

Aldosterone is metabolized mainly in the liver, but the kidney also contributes by 10–15% to the overall metabolism of the hormone. Of the optical isomers, only d-aldosterone is metabolized (182). In man, within 24 hours after injection of tritiated d-aldosterone, about 90% of the total radioactivity given is found in the urine. About 60% of the tritiated metabolites have been identified so far (183), the major part being excreted as glucuronides. Between 15% and 40% of administered aldosterone are excreted as 3α,5β-tetrahydro-aldosterone-3-monoglucuronide (THA-gluc.) (184). THA was first identified by Ulick et al (185), and the conjugation with glucuronic acid in position 3 was reported subsequently by Pasqualini (186). The other main metabolite, aldosterone-18-glucuronide (aldo-18-gluc.), was formerly described as "acid labile or acid hydrolyzable conjugate" or "pH-1 metabolite". It was originally assumed to be a 3-oxo-conjugate (187), but was later characterized by Tait and his associates as an 18-glucuronide (188, 189). Proof of the chemical identity of the 18-glucuronide and the "pH-1 metabolite" is still lacking. About 7–12% of administered aldosterone is excreted as the 18-glucuronide (184, 190, 191). Additional metabolites

found are 5β-dihydro-aldosterone, free and as the glucuronide, and isomers of THA, such as 3α,5α-THA or 3β,5β-THA, both free and glucuronidated (183).

In adrenalectomized rats no mineralocorticoid activities of the THA-gluc. or the aldo-18-gluc. have been found (192, 193), whereas free THA exhibited about 2% of the total aldosterone activity.

Pharmacokinetic studies.—In man the biological half-life of free aldosterone was found to be 30–40 min (194, 195). Metabolic clearance rate was measured as 0.86–1.64 ml/min (196) and similarly as 0.85–1.50 ml/min (197, 198). When calculated for 24 hours, the metabolic clearance rate was 1200–2400 l (19). In patients with essential hypertension a decrease in metabolic clearance rate (867 l/day vs 1480 l/day in control subjects) was found together with a slightly reduced secretion rate and a tendency to elevated plasma concentrations of aldosterone (199).

Biological half-life and metabolic clearance rate are mainly determined by the liver, where 90% of the metabolites are formed and where 90–100% of free aldosterone is extracted. The rate of extraction is directly proportional to the liver blood flow. Nearly all of the THA-gluc., but only 10–30% of the aldo-18-gluc., is formed in the liver, the conjugation of the latter taking place to a major part within the kidney. The kidney is responsible for the formation of about 10–15% of the total metabolites of aldosterone, the extraction rate of free aldosterone being about 20% (14–27%). As in the liver, extraction rate in the kidney is proportional to renal blood flow. A small fraction of aldosterone was found to be metabolized in the intestine and other splanchnic organs (190, 200, 201). It cannot be excluded that in other tissues, where aldosterone acts on sodium transport, such as the salivary glands, the hormone is also metabolized. These fractions, however, are of little, if any, significance in the overall metabolism.

Renal metabolism of aldosterone.—Incubation of slices of human kidney resulted in the formation of THA-gluc. and aldo-18-gluc. (202). When isolated dog kidneys were perfused with tritiated aldosterone, the acid hydrolyzable conjugate appeared in the urine (203). In man, after injection of tritiated aldosterone, the concentration of aldo-18-gluc. in the renal venous blood increased (196). From these observations it was concluded that the 18-glucuronide is formed within the kidney. This assumption received further support from the finding that the acid labile conjugate appeared in the urine at a higher rate than was expected from the renal extraction ratio of that metabolite (184). In addition, in nephrectomized dogs, the plasma concentration of aldo-18-gluc. was reduced (203), whereas removal of the liver affected neither plasma concentration nor urinary excretion of the conjugate (204, 205).

In dogs, intravenously administered tritiated aldo-18-gluc. was only filtered, whereas after injection of tritiated aldosterone the aldo-18-gluc. was also secreted in the proximal tubules. Hence, it was concluded that the glucuronide is formed in the cells of the proximal tubules (206), but other investigators reported that

infused tritiated conjugate was also proximally secreted (207). Despite these discrepancies it is generally assumed that the kidney is the major site of aldo-18-gluc. formation.

In the rat, renal metabolism of aldosterone has not been investigated in detail. However, the overall metabolism is different from that in man and dog, since only a minor fraction of tritiated aldosterone appears in the urine (208). Furthermore, only a small percentage of the urinary metabolites was characterized as glucuronide conjugates (193, 208). On the other hand, biological half-life of exogenous aldosterone was similar to that found in man and dog (209). In sodium-deprived rats, half-life was reduced to 19 min (209).

Renal clearance of aldosterone and its metabolites.—Of the aldosterone present in the glomerular filtrate, up to 14% was claimed to be excreted in the urine (210), but other investigators found lower excretion rates of the filtered aldosterone (184, 196). In dogs, by means of the stop-flow technique, reabsorption of filtered aldosterone was localized in the distal tubules (207, 211). In human plasma, about 37% of THA-gluc. is bound to albumin (212). In man, the clearance of this metabolite corresponds to the inulin clearance (212), and similar results were obtained in stop-flow experiments in dogs (206). On the other hand, the clearance of aldo-18-gluc. exceeds that of inulin, and thus it must also be secreted (210). The plasma protein binding of this metabolite is about 55%, whereas the clearance corresponds to that of PAH (212). Administration of PAH reduced the clearance of aldo-18-gluc., probably by competitive tubular secretion (210).

Further evidence for the fact that aldo-18-gluc. is not only secreted but also formed in the proximal tubular cells is the finding that the renal extraction rates of THA-gluc. and of aldo-18-gluc. are similar (184), whereas the clearance of aldo-18-gluc. is greater. In addition, after intravenous injection of labelled aldosterone, the ratio of tritiated THA-gluc. to tritiated aldo-18-gluc. in plasma is similar in normal and bilaterally nephrectomized subjects (213). Together with the results of stop-flow experiments in dogs (206), these findings support the assumption that aldo-18-gluc. is formed in the cells of the proximal tubules.

In bilaterally nephrectomized patients the biological half-life of aldosterone was reduced to 15–20 min (214). In patients with chronic renal failure the biological half-life was also shortened, and the metabolic clearance rate was increased (197, 198). These surprising observations were explained by the fact that after nephrectomy all aldosterone would be extracted and metabolized by the liver. Since the extraction rate was found to be proportional to hepatic blood flow, it may be assumed that after removal of the kidneys a higher proportion of total blood volume per time unit would pass the liver (214). In chronic renal failure the distribution volume of tritiated aldosterone was increased when it was calculated on the basis of disappearance curves of injected ^{3}H-d-aldosterone. This finding could be interpreted as an increase in distribution volume without an increased metabolic clearance rate (198) or vice-versa (214).

18-Monoacetate of Aldosterone

A substance closely resembling the 18-monoacetate of d-aldosterone (18-MA) has been isolated from heart muscle of various animal species as well as from actively contracting skeletal muscle of cats (215). Initially the substance from heart muscle (HS) was supposed to be identical with 18-MA, but recently it was separated from it by chromatographic methods (215). Lockett and coworkers have claimed that HS is synthetized by heart muscle using d-aldosterone as substrate. In cats, HS and 18-MA both stimulate the secretion of antidiuretic hormone and, in water-loaded rats, induce a dose-dependent antidiuresis (216, 217). In isolated perfused kidneys of cats, HS and 18-MA either increased or decreased sodium and water excretion, the effect depending on specific experimental conditions (218). The significance that the "heart substance" or the 18-MA may have in the regulation of sodium and water balance is not at all clear, and further studies on their production and function are necessary.

Factors Stimulating Sodium Excretion

The present situation in this area is so entangled that it is not possible to review it completely. Various groups have claimed the isolation of a transferable natriuretic factor of renal or other origin, but the differences between the individual factors render it difficult to assess their significance in the control of sodium excretion. The term "third factor", which was coined prematurely (glomerular filtration rate and aldosterone being numbers one and two in determining sodium excretion), has been justly criticized (96, 219) and should no longer be used. The same holds true for the even bolder attribute "natriuretic hormone" (220). However, descriptive terms, such as humoral sodium transport inhibitor (HSTI) (221, 222), are not attractive and certainly not more correct. That a humoral factor may be responsible for the increased elimination of sodium after expansion of intravascular volume was originally claimed by de Wardener et al (97, 223) on the basis of cross-circulation experiments in dogs treated with high doses of 9αFF. Other investigators (224, 225) obtained similar results and ascribed the increased excretion of sodium following expansion of the extracellular space to a substance promoting renal sodium excretion, but contradictory findings have also been reported (226).

Evidence has been accumulated by both in vivo and in vitro experiments that, under conditions of sodium loading or expansion of extracellular fluid volume, humoral factors are produced that promote sodium excretion by inhibiting reabsorption either in the proximal or in the distal parts of the nephron. The disturbing fact, however, is that these factors differ widely in their supposed chemical nature, their possible source, or their physiological activities. The only clear idea is that these agents do not originate from the adrenal cortex, which years ago had been claimed to produce a sodium-excreting factor of steroid nature (227).

Most investigators who support the existence of a factor that promotes sodium

excretion have evidence for its peptide or protein nature. However, some groups found that the molecular weight of their active fractions was of the order of 1000–3000 (228, 229) or even smaller, such as 500–700 (221), while others reported molecular weights of between 10,000 and 50,000 (230, 231). Recently, two natriuretic factors have been described to be present in the urine of saline-loaded man, one of high molecular weight, inducing a delayed and prolonged response, and the other of low molecular weight, which provokes a transient blood-pressure increase and has an immediate effect on sodium excretion and urine flow (231).

Since the early experiments of de Wardener and associates (223), the cross-circulation technique between a donor and a recipient dog has been used to demonstrate the presence of a transferable natriuretic factor. By means of this technique other investigators have obtained evidence for the fact that saline loading or blood-volume expansion stimulates the release of a natriuretic factor (224, 225, 232–235). In a carefully planned joint study three groups of investigators, using four different methods, failed to demonstrate in the plasma of saline-loaded dogs or rats a substance that inhibits sodium transport in the proximal tubules (236). The same authors were unable to confirm claims that such a factor was present in the plasma of dogs that had escaped the sodium-retaining actions of DCA (237). Recently, however, a relationship between sodium excretion and a plasma factor inhibiting tubular sodium reabsorption was claimed in dogs before and during escape (222). Similarly, in the plasma of DCA-treated rats an inhibitor of proximal sodium reabsorption was found (233), but the results have not been confirmed so far, and the substance may share the fate of the factor claimed to be present in dog plasma (236, 238). In comparative studies it was demonstrated that intravenous infusion of isotonic saline into a donor dog was more active in promoting sodium excretion in the recipient dog than was the infusion of blood (234). It was concluded that the production of the hypothetical natriuretic factor was not simply the consequence of expansion of the intravascular volume, but that it was elicited by the intravenous infusion of saline. This, however, is not in agreement with observations that careful control of plasma composition during expansion did not prevent the natriuretic response (98).

In contrast to the various experimental procedures in which a decrease in sodium reabsorption and natriuresis was induced by an expansion of intravascular or extracellular fluid volume, saline loading in subtotally nephrectomized dogs caused a high fractional sodium excretion without an increase in extracellular fluid space (239). Subsequent to a small salt load, these uremic dogs excreted 30% more sodium than dogs in the pre-uremic state. In order to exclude changes in mineralocorticoid secretion, the dogs were treated with 9αFF, and it was found that the elevated rate of sodium excretion per nephron was attributable neither to an elevated glomerular filtration rate nor to variations in endogenous mineralocorticoid activity. It was concluded that factors other than those measured and probably of extrarenal origin may be responsible for the high sodium excretion.

Site of action.—Since an increase in extracellular fluid volume or intravascular volume is accompanied by a reduction in proximal sodium reabsorption, it was supposed that a hypothetical natriuretic factor would inhibit proximal sodium transport. To study the possible mode of action of such a compound, the urinary bladder of the toad was used as an experimental model (221, 229, 240).

Plasma ultrafiltrates from sodium-loaded dogs inhibited sodium transport in the toad bladder for 60–120 min, but the inhibitory activity was no longer demonstrable after ligation of the vena cava inferior (221). However, doubts were expressed as to the specificity of this effect (241). Isolated frog skin (242) and renal tubular fragments (97, 228, 243) were used to demonstrate an inhibitory effect of humoral factors on sodium transport, but the actions observed in vitro could not be confirmed in vivo.

The natriuretic substance of large molecular weight, which has an enhanced and prolonged action, is supposed to affect the distal part of the nephron (230, 231), but evidence for its effect on the distal tubules is still lacking.

Despite the numerous efforts that have been made during recent years to demonstrate the existence of a humoral factor promoting natriuresis, the situation is as enigmatic as it was at the time when, besides a sodium-conserving hormone, another was first claimed to exist that facilitates sodium excretion (223). One must also consider that the natriuresis observed in the whole animal after infusion of isotonic saline could, to a great extent, be explained by a reduction in aldosterone secretion rate and perhaps by adaptation of the renin-angiotensin system.

Although variations in mineralocorticoid activity were eliminated as a cause for an increase in sodium excretion in some experiments (239), in others it was demonstrated that chronic sodium administration may reduce fractional sodium reabsorption in the proximal tubules by diminishing aldosterone production (50). However, the decrease in fractional reabsorption rate was largely compensated in the more distal parts of the nephron. Renin in the kidney was not measured in these experiments, but on the basis of other studies it may be assumed that it was reduced as a consequence of the prolonged salt-loading period.

Renal Effects of Angiotensin

Since renin is produced and released within the kidney, it is obvious to suppose an intrarenal action of the renin-angiotensin system. Studies on the function and significance of endogenous renin and the angiotensin released from specific substrate are handicapped by the scarcity of information on the kinetics of the enzyme-substrate reaction and on the site where the reaction takes place. Most of the investigations have been carried out with angiotensin in various species, including man, but only a few authors have taken into account the state of activity of the renin-angiotensin system and the functional state of the kidney, both of which may influence the effects of exogenous angiotensin. It should also be borne in mind that angiotensin released within the kidney, and exogenous renin or angiotensin, may have different actions on renal functions. Angiotensin, the most potent vasoconstrictor, primarily acts on the renal vascular bed, but there is

also evidence for effects on the tubular system. In view of the close interrelation between hemodynamics and tubular functions it is difficult to distinguish between primary and secondary effects of angiotensin on renal function in the intact animal.

Hemodynamic effects.—Angiotensin induces a dose-dependent direct vasoconstriction in the renal vascular bed, the renal vessels being more sensitive to the polypeptide than other vascular beds (244, 245). Changes in glomerular filtration rate, in renal plasma flow, and in other parameters have been studied under various experimental conditions. When measured by means of inulin or creatinine clearance, a species- and dose-dependent response of glomerular filtration rate to angiotensin was observed. Whereas some authors found glomerular filtration little or not at all affected in rats (246, 247) and rabbits (248, 249), others observed an increase in saline-loaded rats (250). In dogs a reduction in glomerular filtration rate may occur, dependent on the dose (251, 252), whereas in man a decrease in glomerular filtration rate was seen with all doses (253–255). When angiotensin was infused into one renal artery of dogs, glomerular filtration rate and renal plasma flow were diminished (256). In a semi-isolated dog-kidney preparation, angiotensin reduced glomerular filtration rate and renal plasma flow (257). When, after constriction of one renal artery, angiotensin was infused intravenously, glomerular filtration rate of the ischemic kidney increased, independent of the dose within the range studied, the degree of blood-pressure elevation, and the alterations in renal blood flow (258).

Disappearance curves of ^{85}Kr and additional autoradiographic studies showed that infusion of angiotensin II into the renal artery induced a reduction in cortical and in medullary blood flow (259, 260). The vasoconstrictor effect of angiotensin was most prominent in the outer renal cortex.

The intrarenal injection of angiotensin I diminished cortical blood flow, but had no influence on the blood flow of the outer medulla. The effect of angiotensin I was ascribed to its partial intrarenal conversion to angiotensin II. However, a direct effect of angiotensin I could not be excluded (261). In rats, antinatriuretic and natriuretic doses of angiotensin induced a decrease in the clearance of PAH (262). In more recent experiments it was found that the decrease in PAH clearance was not associated with significant changes of PAH extraction (263). However, during angiotensin infusion, an increase in PAH extraction was also noticed (260, 264), which was attributed to a reduced medullary blood flow.

The evidence that a potent vasoconstrictor substance is released within the kidney led to the attractive hypothesis that angiotensin might be the substance responsible for the intrarenal autoregulation of blood flow (265). However, various investigators failed to demonstrate a participation of angiotensin in the autoregulatory process. When renal vasoconstriction was produced by either renin or angiotensin, autoregulation of renal blood flow was maintained (266–268).

When the effects of intravenously and intrarenally administered angiotensin were compared in dogs, evidence was obtained that the inhibition of tubular

sodium reabsorption may be a consequence of changes in the ratio between systemic and renal circulation (252). In rats, however, no relationship was found between the effects of angiotensin on sodium excretion and the changes in blood pressure, renal blood flow, glomerular filtration rate, or filtration fraction (263). It was concluded that natriuresis induced by angiotensin could not simply be the result of altered hemodynamics. In dogs no correlation was found between natriuresis and either the degree of blood-pressure elevation, altered filtration fraction, or the reduction in renal blood flow (258). On the other hand, comparative studies in normal, adrenalectomized, and renal hypertensive dogs revealed that angiotensin can produce a decrease, no change, or an increase in total sodium excretion, the effect depending on the action of angiotensin on the renal vascular bed and consequently on glomerular filtration rate and renal blood flow (269).

Isolated perfused kidney.—The action of exogenous angiotensin and of angiotensin released by endogenous renin has been studied in the isolated perfused kidneys of dogs and rabbits (116, 117, 270–272). The increase in renal vascular resistance observed during perfusion of the kidney with blood has been ascribed, at least in part, to the reaction of endogenous renin with its substrate (270, 271). In order to prevent the renin-substrate reaction, kidneys were perfused in a recirculation system by a substrate-free medium consisting of a red-cell suspension in an albumin-electrolyte solution (116). Addition of substrate to the perfusion medium increased glomerular filtration rate and filtration fraction, which indicates vasoconstriction of the efferent arterioles. These effects were abolished in renin-depleted kidneys taken from rabbits that had been pretreated with DCA and salt (116).

In rat kidneys perfused with a renin substrate-free medium in a nonrecirculated "open" system, addition of substrate resulted in an increase in intrarenal resistance, which has to be ascribed to the rapid interaction of endogenous renin with exogenous substrate and the release of angiotensin (273). Infusion of angiotensin I or angiotensin II elicited a similar intrarenal vasoconstriction. Administration of a converting-enzyme inhibitor (SQ 20881) reduced the effect of angiotensin I, which suggests that converting enzyme is present within the rat kidney (K. Hofbauer et al., in preparation). In dogs, other investigators also presented evidence of an intrarenal conversion of angiotensin I to angiotensin II (274, 275).

When small doses of angiotensin were given to the substrate-free perfusate of rabbit kidneys, glomerular filtration rate and filtration fraction increased similarly as after the addition of substrate, and absolute sodium reabsorption rose (276). Higher doses of angiotensin reduced glomerular filtration rate as well as absolute sodium reabsorption (K. Hofbauer et al., in preparation). Obviously, in the low-dosage range, angiotensin acts predominantly on the efferent arteriole, inducing an increase in glomerular filtration rate and filtration fraction, whereas, with higher doses, additional constriction of the afferent arterioles diminishes

glomerular filtration rate. Variations in sodium reabsorption under these conditions may be a consequence of changes in renal hemodynamics rather than in tubular function (276). In rabbit kidneys perfused with oxygenated Krebs solution a similar effect of angiotensin on the efferent arteriole was observed. This effect is apparently specific, because epinephrine acted on the afferent arteriole (272, 277).

From studies in the bullfrog (278), in dogs, and on isolated perfused kidneys of rabbits a primary effect of angiotensin on the efferent arterioles can be assumed (117, 272, 277), which, in addition to inducing changes in glomerular filtration rate, may contribute to alterations in sodium excretion. Constriction of the efferent arterioles is followed by a rise in capillary pressure proximal to, and a reduction distal to, the constriction. As a consequence, proximal tubular reabsorption of sodium may be enhanced. Such a hypothesis is supported by the observation that the intra-aortic infusion of acetylcholine elicits natriuresis (279). Acetylcholine, by dilating the efferent arterioles and subsequently raising the peritubular capillary pressure, may diminish proximal tubular reabsorption of sodium. A reduction in tubular sodium reabsorption was also observed when the hydrostatic pressure in the peritubular capillaries was increased by renal venous constriction (280).

Effect on renal sodium transport.—The question whether angiotensin has a direct influence on tubular sodium transport cannot yet be answered definitely, but there is increasing evidence that variations in sodium excretion produced by angiotensin may be explained exclusively by hemodynamic mechanisms. When angiotensin was applied in various concentrations to either peritubular capillaries or the tubular lumen, micropuncture studies gave no evidence for a direct effect of angiotensin on sodium transport in the proximal tubules and in Henle's loop (281). On the other hand, an effect on sodium reabsorption in distal tubules was observed when the peritubular capillaries were perfused with angiotensin, but not when angiotensin was in the tubular fluid (282). With high intravenous doses of 0.2–1.5 γ/kg/min, sodium concentration in the distal tubular fluid increased, and, in the upper dosage range, diuresis and natriuresis resulted. The data obtained in dogs by means of the stop-flow technique were suggestive of a direct inhibitory effect of angiotensin on sodium transport in the distal tubules (256).

In isolated perfused proximal tubules from rabbit kidneys, angiotensin had no effect on fluid absorption rate (283). In other experiments it did not influence electrolyte or water content of isolated rabbit tubular cells, whereas ouabain significantly altered cell composition.

Obviously angiotensin has no effect on sodium-potassium dependent ATPase and does not directly act on ATP-linked sodium transport (284). Recently, however, it was claimed that low doses of angiotensin stimulated the activity of the sodium pump in renal cortex slices of rats, and that this activity was not inhibited by either ouabain or the absence of potassium (285). It was suggested that angiotensin acts on a sodium-pump mechanism that is potassium-independent and

resistant to ouabain. Since these observations are in contrast to most other findings, it is necessary to confirm them by means of other experimental techniques.

Effect of angiotensin on transepithelial transport.—Simplified models, which have been used successfully in the investigation of electrolyte transport, such as the toad bladder or the frog skin, were also applied to studies on angiotensin. In contrast to vasopressin, which increases the permeability for sodium at the apical surface of epithelial cells of the toad bladder or skin (47), angiotensin showed no such effect. Angiotensin, in concentrations that were much higher than those of vasopressin, was applied to either the mucosal or the serosal side of the epithelial preparations of the bladder, skin, or colon of *Bufo marinus*, but had no influence on electrolyte transport (286–288). However, an action of angiotensin on sodium transport by the frog skin was reported by McAfee & Locke (289). Fluid transfer has also been studied by means of isolated everted sacs of jejunum, ileum, and colon obtained from adrenalectomized and nephrectomized rats (290, 291). Angiotensin stimulated fluid transfer in everted sacs of the three intestinal regions and exhibited a dose-dependent action in stripped colon-sac preparations, stimulating fluid transfer in low concentrations and inhibiting it in high concentrations (291). In the rabbit gall bladder, high concentrations of angiotensin reduced fluid transfer significantly (292). Despite these observations, which apparently demonstrate an effect of angiotensin on fluid and electrolyte transport, it is obvious that this effect is not comparable in either degree or specificity with the actions of vasopressin or aldosterone observed in various epithelial preparations.

The data obtained from both in vitro and in vivo experiments render it improbable that angiotensin has a direct effect on tubular sodium transport in the kidney. Natriuresis and sodium retention, both observed during the administration of angiotensin, may be explained by hemodynamic mechanisms that indirectly affect tubular function.

Determinants of the renal response to angiotensin.—In most animal species, angiotensin has different effects on diuresis and natriuresis, depending on the dose and, to a certain degree, the state of sodium balance. It has been claimed that rats respond to angiotensin exclusively by an increase in fluid and sodium excretion (246), but subsequent studies revealed that small doses produced antidiuresis and antinatriuresis, whereas high doses resulted in a biphasic reaction consisting of an initial reduction followed by a marked increase in sodium and water excretion (247). Similar dose-dependent responses have been found in other species, such as the dog (252, 293–296) and the rabbit (248, 249, 297). In man, antidiuresis and antinatriuresis were reported in most cases (253, 298–300), but occasionally an increased sodium excretion was described (295).

The state of sodium balance may affect the response to angiotensin. Sodium depletion (which stimulates the activity of the renin-angiotensin system) lowered

the threshold to the natriuretic effect of angiotensin in rabbits, whereas in salt-loaded animals (which have a low endogenous renin activity) an antinatriuretic effect was prevalent (301). Comparable observations were made when angiotensin was infused in rats bearing a clip on one renal artery. In the clamped kidney, which had a high renin content, angiotensin induced natriuresis, whereas the contralateral (renin-depleted) kidney responded with sodium retention (302). In renal hypertensive rats, angiotensin infusion increased sodium excretion only in the presence of a contralateral, untouched kidney. An "exaggerated" natriuresis subsequent to angiotensin infusion was demonstrable in saline-loaded hypertensive rats, independent of the presence of a contralateral kidney (250). In other experiments no consistent effect of angiotensin on sodium and water excretion was seen in dehydrated or nondiuretic rats, but after loading with isotonic saline or a hypotonic solution a diuretic and natriuretic response was obtained (303). It was concluded that a certain degree of initial fluid retention is a prerequisite for the diuretic and natriuretic effects, and that angiotensin might act by enhancing the diuretic and natriuretic response to an expansion of the extracellular space. Such a claim is, however, not in agreement with the reduced natriuresis found in salt-loaded rabbits (301), but different experimental conditions may be responsible for the discrepancy.

Indirect action of angiotensin.—Since angiotensin stimulates aldosterone secretion from the zona glomerulosa of the adrenal cortex, it may indirectly influence sodium transport in the distal tubules. Hence the finding of an increased sodium excretion after the administration of angiotensin appears to be paradoxical in view of the role ascribed to the renin-angiotensin system in the maintenance of sodium balance. In this respect the reduced sodium excretion seen after small doses of angiotensin would be a more adequate response, but an additive or synergistic effect on tubular sodium transport is in no way established. Besides stimulating the release of aldosterone, small doses of angiotensin also enhance the release of antidiuretic hormone (ADH) (304), which may contribute to the overall effects of the renin-angiotensin system on sodium and water balance. Infusion of angiotensin into the carotid arteries resulted in a more marked stimulation of ADH release than did intravenous administration (305). The effect of angiotensin on the secretion of ADH was not mediated by altered plasma osmolality or sodium concentration, but by a direct effect on some area in the brain related to the release of ADH. In cats, microiontophoretic application of angiotensin II to supraoptic neurosecretory cells was followed by a release of ADH (306). In rats, centrally administered (into one lateral ventricle) angiotensin II elicited a primary antidiuresis and a secondary increase in urine flow and electrolyte excretion, possibly via release of ADH (307). The stimulation of other hormonal systems, and even blood-pressure elevation in itself, may modify the direct intrarenal action of angiotensin in the whole animal, but not in the isolated perfused kidney, which therefore is an excellent model for studying angiotensin, provided that the perfusion technique allows the maintenance of adequate renal function.

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