RENAL PHARMACOLOGY[1]

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Although a great deal of work has been reported from various laboratories, the past year has seen no major advance in an understanding of the basic processes by which substances are moved selectively across the tubular epithelium.

This is not to say that no useful insights have been obtained on aspects of renal function. The countercurrent mechanism that is responsible for the production of hypertonic urine in the mammal seems now well established. Gottschalk (117) has recently reviewed carefully the details of the process as it has been elucidated by micropuncture and renal slice studies. The solute gradient of the kidney was diminished under the conditions of mannitol loading in normal or diabetes insipidus dogs (3) and in rats (2). The gradient was least in the normal hydropenic state with mannitol loading, rather than in dogs with diabetes insipidus. There was an indication of a difference in the rates of urea and water movement (134). The potassium gradient was unaltered by these conditions.

That there is an active transport of sodium seems, likewise, thoroughly based in fact. Giebisch & Windhager have reviewed critically the experiments, largely based upon studies of single nephrons, initially in *Necturus* and later in mammals, that established the primacy of a sodium pump in the transfer of sodium chloride and water (111, 307). A comparison of these reviews with an earlier one (110) reveals the mass of supplementary and clarifying data that are now available to support the concept. The active reabsorption of potassium has been established in comparative studies in the dog, rat, and *Necturus* in various states of potassium loading (292). Although chloride transport appears to be largely passive, the divergence between flux data and transtubular potentials is cited as evidence that a component of active transport may be present, at least in the rat (226). The absence of a one-to-one relation between sodium and potassium in the distal tubule under appropriate conditions of pretreatment and the existence of an electrical gradient which is more than adequate to transport potassium passively led Giebisch & Windhager (111) to conclude that no carrier-coupled sodium-for-potassium exchange needs to be postulated at the luminal cell membrane of the distal segment. Alternatively, sodium transport may indirectly influence potassium secretion as it affects the intracellular potassium concentration or the net electrical gradient across the epithelium (111).

Although much useful detailed data are now available to describe the

[1] The survey of the literature pertaining to this review was concluded in May 1965.

sites, magnitudes, and direction of movement of the various substances as transported by the kidney, there is still no clear indication of (*a*) the steps whereby the energy is provided for the process of active transport or (*b*) how the actual entrance of the substance across the interfaces of the epithelium is accomplished. Recent studies have demonstrated a good correlation between oxygen consumption and sodium reabsorption (*76*, 106, 160, 166, 276). Renal medullary tissue has long been known to be capable of converting glucose anaerobically to lactate, even in the presence of oxygen. The relatively poor blood and oxygen supply imposed by the countercurrent pattern of the vasa recta suggested a teleological relationship and a possible importance of glycolysis in sodium transport in the loop of Henle.

Thurau (278), in a review of renal hemodynamics, observed that, although only 6 per cent of the renal blood flow passes through the medulla, the total flow per unit tissue weight is actually relatively large, being about 15 times that of resting muscle and 50 per cent of that in the brain; hence the oxygen supply is not negligible in the medulla. Small alterations in medullary blood flow might be important in regulating the concentrating mechanism. This may assume importance in interpreting experiments in which ureteral clamping or arterial occlusion is performed (86, 254). The medullary anoxia that may be induced during the occlusion of a stop-flow experiment may be of critical importance.

Cahill and his associates (172) and McCann (182) have stressed the importance of oxidative processes in the renal medulla. Although glycolysis to lactate occurs to a greater extent in medullary than in cortical tissue, oxygen uptake and carbon dioxide formation by medullary tissue are considerable. The oxidative process, as well as glycolysis, increases with increasing osmolality of the medium. Kean & Davies (143, 144) had earlier associated a high glycolytic rate with the high interstitial osmolality that is found in the medulla. As Lee et al. (172) had found in the rabbit, Bernanke & Epstein (28) showed similarly in dog renal slices that oxidation of glucose proceeds readily in the medulla. They attributed to this process the energy required to operate the renal concentrating mechanisms. Earlier, Cohen (61) had noted the large and variable respiratory quotient of kidney tissue and had proposed that the energy for transport was delivered in part to the kidney in the form of metabolic intermediates, as α-ketoglutarate, that were utilized anaerobically. More recently, he has proposed that the weak acid transport system commonly associated with *p*-aminohippurate (PAH) may in actuality be the mechanism whereby the essential metabolite, α-ketoglutarate, is transported to its metabolic site: "It is suggested that the general and perhaps primary function of the PAH transport system is to move specific substrates to sites of dissimilation in the liver and kidney" (255). The fact that α-ketoglutarate is transported (14, 255) and also utilized, as well as other Krebs cycle intermediates (180), and the fact that the kidney can either form or utilize glycogen from glucose under various conditions, make interpretation of the various observations more difficult

(171, 182). The work of Kessler (106) is interpreted to implicate the electron transport chain as a component of the system that provides energy for active sodium transport. Whichever of the basic biochemical processes may be involved, some storage of energy in high energy phosphate bonds is likely to occur. As a penultimate stage in the delivery of energy to the sodium pump, recent attention has been given to a membrane adenosinetriphosphatase that is operationally defined as a ouabain-sensitive Na-K-dependent system. At the present time, however, the enzyme system is as yet too poorly defined biochemically and functionally to be accepted without further investigation as the sole subcellular basis for electrolyte transport.

Two illustrations may be drawn from the recent literature to demonstrate the importance of the biochemistry of membrane permeability, in contradistinction to the biochemistry of intracellular processes. Phlorizin has been known for many years to inhibit tubular reabsorption of glucose, inducing glycosuria in the mammal. It was initially proposed that phlorizin interfered in the active transport of glucose, probably by interference in the phosphorylation process. This was, in fact, found to be true in yeast, but later established not to be true in mammalian cells. As a result of careful studies by Lotspeich (179) at extremely low concentrations of phlorizin (10^{-7} M), it appears that phlorizin acts by decreasing membrane permeability, rather than by an interference at an intracellular or phosphorylating site (47). The transport of water across the amphibian skin or bladder is facilitated by vasopressin or theophylline (22, 127, 285). Orloff & Handler (210) showed that 3′,5′-AMP has a similar effect. According to their view, vasopressin facilitates the formation of 3′,5′-AMP by the enzyme adenyl cyclase. Theophylline is believed to exert its effect by inhibiting the enzyme diesterase which degrades 3′,5′-AMP (89). The action of both vasopressin and theophylline, but not that of 3′,5′-AMP, may be suppressed with cysteine (209). In the presence of cysteine, the cell membrane appears to be impermeable to vasopressin and theophylline, but not to 3′,5′-AMP. In the mammalian kidney, unlike the amphibian bladder, vasopressin and theophylline have dissimilar, not equivalent, effects on urine flow.

Transport of Organic Substances

The transport of organic substances has received less attention than the pathways for transport of sodium and other inorganic ions. Two major papers (79, 297) have reviewed the transport of organic acids from rather different viewpoints. Weiner & Mudge (297) have clarified a three-component mechanism, involving (*a*) glomerular filtration, (*b*) active proximal tubular secretion, and (*c*) passive back-diffusion. Whereas PAH is presumably handled solely by (*a*) and (*b*) and shows net secretion, other compounds that also participate in (*b*) may have net clearances less than glomerular filtration rate because of participation of component (*c*). The importance of lipid solubility and ionization constants for participation of the compound in the latter component of transport has been reviewed

(294). In the view of Weiner & Mudge (297), back-diffusion is demonstrated if the renal clearance is altered by changes in urine pH or in the urinary flow rate. Very weak or very strong acids exist predominantly in the un-ionized or completely ionized form at physiological pH; the clearance will not be affected importantly by pH. Likewise, moderately weak acids that are extremely lipid-soluble or extremely lipid-insoluble (e.g., PAH) will have excretion rates independent of changes in urine pH. Among a group of moderately lipid-soluble compounds of intermediate pK_a, however, back-diffusion will decrease and the renal clearance increase as pK_a or lipid solubility decreases (280, 299). No satisfactory definition of lipid solubility is proposed. Although Weiner & Mudge (297) refer to partition coefficients between chloroform and water, they apparently used 0.15 M HCl as the aqueous phase to assure that the acids would be essentially in the un-ionized state (294).

In their review, Weiner & Mudge apply similar reasoning to the transport of weak bases. They caution that, useful as the three-component pattern may be as a generalization, the possibility that an active reabsorptive mechanism may be operative in a given instance should not be neglected.

Despopoulos (79) has emphasized those structural features which are common to compounds that appear to be actively secreted in the same manner as PAH. The magnitude of the undertaking is underlined by the compilation of 154 compounds that meet several or many of the criteria for participating in this transport process, e.g., transport against a gradient, saturation of transport, enhancement by acetate, depression by anoxia, metabolic inhibitors or probenecid, mutual inhibition by PAH or other substrates—as these are measured *in vivo* in renal clearance experiments or *in vitro* in kidney slice accumulation studies. A three-point model containing an ionizable end and a potential oxygen-hydrogen bridge moiety is evoked, the distance between the points of attachment corresponding to those in an extended or folded structure of PAH. It is of interest that the model permitted the prediction that 5-aminoorotic acid should be a substrate for the system. Certain dicarboxylic acids should participate in the PAH system, and might in this way depress PAH accumulation in the slice, as observed by Cross & Taggart (68); α-ketoglutarate may be transported by this system (14, 62, 255). Although iodopyracet (Diodrast) is an analogue of an acceptable structure, iodopyracet itself does not conform to the general structural requirements. More important than the absolute success of the attempt, however, is the fruitful evaluation of biological and structural data that derive from the survey of the diverse anions that appear to be implicated as substrates in the system.

The key role played by probenecid in an interpretation of the weak acid transport mechanism was noted by both authors. Relatively few of the parameters of the transport of probenecid itself have been measured. Beyer (38) noted originally that probenecid was only partially bound to plasma

protein and was, therefore, ultrafilterable, but its excretion was so low as to indicate complete reabsorption. Later (31), with a homologous series of N-alkylated *p*-carboxybenzenesulfonamides, all of which inhibited PAH secretion in the dog, he showed that the net clearances decreased with increasing chain length. Although the clearances of intermediate members were less than glomerular filtration rate ($0.05 \times$ GFR for $R = C_2H_5$; $R' = C_3H_7$), their clearances were all depressed at high plasma levels ("self-depression") and by PAH, indicating a three-component pattern of filtration, secretion, and reabsorption. The clearance of probenecid was too low to measure, warranting no conclusion concerning the secretory aspect of its transport. This aspect was uncovered under conditions of alkaline urine and high urine flows (in excess of 15 per cent of GFR) in the dog (299) and in man (75). Under these conditions, net secretion was shown, even without introducing the always somewhat questionable correction for plasma binding.

Braun & Schniewind (43) showed that probenecid-S^{35} was accumulated by the guinea pig slice and that the accumulation could be partially blocked by an equal concentration of bromcresol green. Whether or not the *in vitro* accumulation of probenecid is affected by PAH, acetate, or dinitrophenol is not known, nor is the extent of binding to tissue or the rate of efflux from the slice. *In vitro* probenecid inhibits the uptake of many compounds, but the kinetics of its action relative to other competitive inhibitors has not been well quantitated. Benzmalecene and related compounds (13) inhibit PAH uptake at lower concentrations than does probenecid, suggesting that they may have more affinity for the system.

Whether the relative affinity of probenecid exceeds that of other agents for the secretory pathway cannot be accurately ascertained *in vivo,* because of the unknown effects of such extraneous factors as metabolism, storage at active subcellular sites, or binding to plasma protein (297).

The merits of probenecid as an inhibitor of secretion of weak acids rest in part on its long duration of action in animals (38) and in man (75). Beyer (38) noted that detectable amounts were present in the plasma of dogs for more than 24 hours, and Dayton (75) measured a plasma half-life of probenecid in man of 6–12 hours following a single intravenous dose of two grams. Inasmuch as less than 5 per cent of the dose was excreted in the urine, metabolism of the drug very likely occurs, but no products have been identified except the presence of glucuronide in urine (38, 245). It may, like other acids, be secreted slowly in the bile (9, 247). Whether its duration is caused solely by accumulation of the probenecid by tubular reabsorption or whether it is bound avidly at subcellular sites in the secreting regions has not been established.

If active reabsorption or protein binding at cellular sites occurs and is pH-dependent, the competition of probenecid for the PAH transport system cannot be assumed to be specific. If the transport of probenecid does

involve only active secretion and passive reabsorption, the ability of probenecid to depress PAH clearance nearly equally well in acute acidosis or alkalosis indicates that the secretory component is not greatly altered by changes in urine pH (297).

Despopoulos (80) has questioned the causal relation between renal clearance and lipid solubility of the probenecid analogues, noting that renal clearances correlate equally well with plasma binding, the least bound compound having the higher clearance, and that binding, unlike lipid solubility, is not inevitably related to increasing alkyl chain length. The efflux rates of a diverse group of compounds appeared to be independent of the lipid solubilities.

Kinetic studies of the effect of inhibitors, including probenecid, on efflux of PAH from goldfish (152), flounder (151), rabbit (132), and dog (95) kidney *in vitro* have shown that probenecid and other inhibitors may have a biphasic action in PAH efflux, indicating that probenecid may inhibit an active outward-directed route of PAH transport. Farah (95) has suggested that the accumulative "pump" is intracellular and that the latter is at the cell membrane. That dinitrophenol had a similar effect to probenecid, and that uptake of dinitrophenol was inhibited in rabbit tubule fragments by probenecid, suggest that dinitrophenol may also be a competitive inhibitor for the transport process, in addition to its generally recognized action as a metabolic inhibitor (132). The runout of N-methylnicotinamide, by contrast, showed only a slight active component (94).

It may be an oversimplification to separate transport of organic substances into two discrete mechanisms, one for acids and one for bases. Rennick (229) has recently reported that norepinephrine and dihydroxyphenethylamine (DOPAmine) are transported by both mechanisms in the chicken. In an earlier paper, Rennick (231) had shown that epinephrine was secreted by the renal tubule in the chicken and that this secretion was inhibited by probenecid but not by cyanine No. 863, a dye which is an inhibitor of the transport of many bases (213). Although not able to demonstrate blocking of epinephrine transport by PAH or bromcresol green, she concluded that epinephrine was transported by the acid pathway. Rennick (229) has recently reported that norepinephrine is also secreted in the chicken, but, unlike epinephrine, its transport is blocked by both probenecid and cyanine No. 863. She concluded that norepinephrine was transported by both the acid and base pathways. DOPAmine was shown to have similar behavior (229, 243). In chicken renal slices, uptake of both epinephrine and norepinephrine was inhibited by PAH, bromcresol green, probenecid, and chlorothiazide, as well as by cyanine No. 863. The *in vivo* experiments may be more meaningful, as metabolic stability of the substrates was monitored, using chromatographic techniques (230). The metabolism of catecholamines is not a simple phenomenon (197, 301).

The transport of uric acid has been reinvestigated by Beechwood,

Berndt & Mudge (21) in the rabbit. Using a stop-flow technique, a proximal secretory site was observed in 45 of 58 experiments and a reabsorptive site in three animals. Net clearance was usually less than filtration rate; in some experiments net reabsorption was shown in free-flow samples, but a paradoxical secretory peak occurred, suggesting that transport characteristics were altered under stop-flow conditions. There was considerable variation among animals in the urate/inulin peak and in the free-flow values, apparently unrelated to sex, urine flow, urate plasma level, or glomerular filtration rate. There was no evidence for distal transport. It was concluded that bidirectional transport occurs in a more proximal region and that the distal region is more or less impermeable to urate. Chlorothiazide, lactate, exogenous creatinine, and ouabain (29) increased the proximal urate peak, and probenecid reproducibly decreased the net proximal transport to produce a net reabsorptive effect. Pyrazinoic acid caused an increase in net secretion in 8 of 15 animals and no change in the others. Salicylate had no effect at 30 mg/kg. It should be noted that the drug effect on free-flow clearance of urate was minimal. Since chlorothiazide, probenecid, and ouabain inhibit urate uptake in rabbit kidney slices *in vitro* (29, 219), their contrary actions *in vivo* require further clarification.

The kidney acts to conserve amino acids by tubular reabsorption mechanisms. The capacity to absorb neutral amino acids is very great; for dibasic amino acids, specifically arginine and lysine, a maximum capacity (Tm) was demonstrated (39). The amino acids have been separated into groups, within which competitive reabsorption may occur but among which no competition is demonstrated, evidence being derived variously from analogy with intestinal transport data, from renal studies, and from clinical studies of rare genetic disorders of amino acid metabolism (191, 192, 196). Webber (293) has more recently shown competition between glutamic and aspartic acids in the dog, and Scriver, Efron & Schafer (252) showed that proline had a Tm in man, and that its excretion could be depressed by hydroxyproline. As a result of interest in the familial disorder cystinuria, Rosenberg, Segal and co-workers (104, 238) studied the transport of lysine, arginine, ornithinine, and cystine, using a renal slice technique similar to that of Cross & Taggart (68). Active transport was clearly shown among the former but not with respect to cystine. The implications of these findings to cystinuria have been summarized by Bartter (19). Rosenberg (253) extended the slice technique to a study of the transport of lysine, phenylalanine, and histidine, showing that the accumulation was enhanced by phlorizin. They interpret the data to show that phlorizin acts on the cell membrane to inhibit passive efflux of the amino acids and does not affect active transport. The ionic requirements for uptake of amino acids by kidney slices suggest that the Na-K-dependent ATPase may be involved in transport (105).

Ouabain and Sodium Transport

Koefoed-Johnsen & Ussing (157) have proposed that sodium transport by frog skin is dependent on potassium exchange at the inner surface of the membrane, and that cardiotonic aglycones decrease sodium transport in that organ as measured by the *in vitro* short-circuit method, presumably by inhibiting the Na^+-K^+ exchange (156, 157). When injected directly into the renal artery, ouabain or strophanthidin exerts a direct effect to decrease sodium, chloride, and water reabsorption [Farber, Alexander & Earle (97); Tsunemi (281); Tanabe et al. (273)] and to decrease hydrogen and potassium secretion, accompanied by an increased urinary pH in chicken clearance experiments [Orloff & Burg (208); Cade et al. (48)]. Thus, their renal actions have been considered to be similar to their effect on the sodium "pump" of nerve, muscle, and erythrocytes. Wilde & Howard (305) and White & Rolf (302) have interpreted their stop-flow experiments to be consistent with a natriuretic action of ouabain at both proximal and distal portions of the nephron in the dog.

These compounds inhibit a sodium-potassium-magnesium-dependent adenosinetriphosphatase (ATPase) found in the membrane fraction of kidney homogenates (41, 150, 260, 303), in the ghosts or membrane fragments of erythrocytes (85, 223), in nerve (261), and in heart (125, 283). Skou (262) has reviewed the evidence relating this membrane ATPase activity to the Na^+-K^+ membrane "pump." However, this mechanism of membrane ATPase inhibition may not account completely for cation transport. The renal diuretic and the cardiac inotropic effects are inversely related dosagewise to ATPase inhibition. Kleinzeller & Knotkova (155) demonstrated that, whereas substantial concentrations of ouabain did inhibit a portion of the extrusion of sodium or lithium and water from previously loaded renal cortical slices, dinitrophenol more completely inhibited the egression of electrolytes and water. Palmer & Nechay (211) reported that the stimulation of chicken kidney microsomal Na^+-K^+-dependent ATPase by low concentrations of ouabain and inhibition by higher concentrations were correlated directly with a reduction in sodium, potassium, chloride, and water output at low dosages infused into the intact renal portal system of the bird, and an increased output of water and electrolytes as the dosage was increased. Conversely, in the heart, low (inotropic) concentrations of such cardiotonic agents stimulated ATPase activity and induced a net gain in intracellular concentration of K^+ in isolated atrial strips, whereas higher concentrations caused a loss in intracellular K^+ (122, 153, 232, 282). Whereas other agents, such as the thiazides, ethacrynic acid, and the organomercurials have little or no demonstrable effect on either *p*-aminohippurate (PAH) or N′-methylnicotinamide (NMN) transport by the tubules at submaximal saluretic dosages, ouabain depressed both PAH and NMN tubular secretion by the chicken [Nechay & Pardee (202)], and strophanthidin inhibited PAH accumulation by rabbit renal slices [Burg & Orloff (46)]. Like

the natriuretic effect of these compounds (48, 208), their inhibition of PAH or NMN is reversed or antagonized by increased potassium concentration. While membrane ATPase may not be directly implicated, its augmentation of cytoplasmic glycolysis, reported by Jones, Lockett & Landon (137, 138), or some such interaction apparently does contribute to the energy requirements for transport of organic acids and bases. Dinitrophenol, which serves to uncouple oxidative from phosphorylative processes, likewise inhibits both NMN (228) and PAH (37) secretion, but it does not inhibit sodium reabsorption even when administered into the renal artery of the dog (199); this seems consistent.

Certain other observations which may or may not be related should be pointed out in closing this section on ouabain and its analogues. Orloff & Burg (208) indicated that the pH of urine increased when strophanthidin was administered to the chicken and that the reciprocal relationship between H^+ and K^+ secretion seen when carbonic anhydrase inhibitors are administered to induce natriuresis did not attend the strophanthidin effect. Kagawa, Baran & Krol (139) reported that when ouabain was injected into adrenalectomized rats it induced an increased excretion of sodium, potassium, and chloride ions. Whereas spironolactone was without effect on the substantial kaliuretic action of ouabain, the aminopteridine triamterene inhibited the kaliuresis. Burg & Orloff (46) could demonstrate no effect of mineralocorticoids, including aldosterone, on the strophanthidin inhibition of PAH accumulation in renal slices from the normal rat or the adrenalectomized dog.

Organomercurials

Since the original disclosure of the diuretic propensity of the antisyphilitic organomercurial Novasurol by Saxl & Heilig (244), the literature on the site and mode of action of these agents has been as conflicting as the recognition of their utility has been uniform. It is clear that they act on the kidney, as Govaerts (120) transplanted a kidney during mercurial diuresis to a control dog, whereupon that organ continued to diurese. Pitts (215) repeated more definitively an earlier experiment by Bartram (18), wherein he injected Hg^{203}-labeled chlormerodrin into one renal artery and observed a diuretic effect first on that kidney and later an increased urine flow from the contralateral organ.

That they act on the proximal portion of the tubule seems certain, as is the interpretation of other data that their action is not confined to this area. Giebisch (108) demonstrated that mercurials reduced the potential measured across the proximal tubules, which potential is attributed to the active transport of sodium and accounts for the passive back-diffusion of chloride [Giebisch (109); Windhager & Giebisch (306); Giebisch & Windhager (112)]. From his studies on active sodium transport, electrical potential, and chloride permeability of the toad bladder, Jamison (135) concluded that his results were consistent with a direct depression of active sodium transport by mer-

curials, rather than a decrease in permeability to chloride. Vander et al. (287) and White & Rolf (302) arrived at a proximal site of action from their stop-flow experiments in dogs. Indeed, the histochemical demonstration of mercury in the proximal cells [Timm & Arnold (279)] and the reduction in protein-bound sulfhydryl concentration especially therein [Cafruny & Farah (49)] do not necessarily relate to site of action, as Farah & Kruse (96) showed reduction of the sulfhydryl reaction to occur also with certain nondiuretic mercurials. Miller & Farah (195) showed that Hg^{203}-labeled *p*-chloromercuribenzoate was accumulated in the cortex, but essentially not in the medulla of the kidney, in confirmation of prior work (121) and consistent with the findings for chlormerodrin (42). The localization of this accumulation in the proximal tubules gains credence from the observation by Campbell (52) that probenecid could block the accumulation and the diuretic action of a mercurial in the chicken, and from the report of Miller & Farah (195) that *p*-chloromercuribenzoic acid, which is not a diuretic agent, could block or reverse competitively the accumulation of Hg^{203}-chlormerodrin and its diuretic effect in the dog. By the conventional clearance analysis of the effect of meralluride, mercaptomerin, or mersalyl, Porush et al. (222) have reaffirmed earlier reports with regard to a proximal site of action, but, whereas the mercurials increased osmolar clearance in man, they had no consistent effect on the free water clearance (C_{H_2O}) or the distal back-diffusion of water ($T^c_{H_2O}$). Both C_{H_2O} and $T^c_{H_2O}$ were increased by the prior or subsequent coadministration of an osmotic diuretic agent. They considered their data to be consistent with the possibility that the organomercurials exert an inhibitory effect beyond the ascending limb of the loop of Henle (116, 222). The fact that mersalyl-theophylline blocks the kaliuretic effect of acetazolamide (26) and chlormerodrin inhibits the chlorothiazide-induced kaliuresis (217) also points to a site of action in the distal portion of the nephron, including perhaps the upper collecting tubules.

There have been two thoughtful interpretations as to which is the active form of an organomercurial diuretic agent. Kessler, Lozano & Pitts (149) support the position that the intact molecule is active, and Levy, Weiner & Mudge (174) have considered that a cleavage of a carbon-mercury bond is needed to form an active mercury ion, a view expressed earlier by Sollmann, Schreiber & Cole (263). The Kessler et al. view is a two-receptor site, one of which is probably sulfhydryl and capable of combining with mercury, the other binding a hydrophylic group placed three carbon atoms from the mercury. The Levy et al. proposal requires a sulfhydryl site and an amino or carboxyl site, both capable of combining with the bivalent mercury. Weiner, Levy & Mudge (296) have studied the relationship of chemical structure to acid-sulfhydryl lability, to diuresis as influenced by pH, to rate of excretion, etc. within a series of organomercurials. They found that all the diuretic organomercurials were acid-labile, giving rise to destruction of the carbon-mercury bond, and all the acid-stable compounds

were nondiuretics. Not all acid-labile compounds were diuretic. The difference between acid-labile diuretics and nondiuretics was correlated with the extent the compound was excreted; those being substantially excreted were diuretics. Since organomercurials are almost completely bound on plasma proteins [Calesnick & Wase (51)], tubular secretion contributes predominantly to their excretion [Kessler et al. (147)]. Thus, Weiner, Levy & Mudge (296) concluded that the correlation between acid lability and diuretic properties strongly suggests that it is the bivalent mercuric ion which combines with the appropriate receptor within the kidney to induce diuresis. Shifts in acid-base balance had no effect on the rate of excretion of the organomercurials, just as Weiner et al. (295) had observed earlier that changes in pH did not affect the loss of mercury from the renal parenchyma whether or not its elimination was accelerated by dimercaprol. Whereas Calesnick & Wase (51) claimed a dissociation between Hg^{203}-mercaptomerin excretion and diuretic response, Levy, Calesnick & Wase (173) re-examined the time course, using the same compound, and concluded there was a definite relationship between diuresis and Hg^{203}-mercaptomerin excretion. The association of mercuric ion formation with diuresis has recently received strong support from the technique employed by Clarkson, Rothstein & Sutherland (60) to show that in the rat the time course for the breakdown of Hg^{203}-chlormerodin to yield mercuric ion correlated convincingly with both the quantitative aspects, the onset, and the duration of diuresis.

Present reports pertaining to cellular localization and action of organomercurials are interesting and merit further exploration. Bergstrand et al. (23) found methyl-mercury^{203}-dicyanodiamide fixation in the kidney to be localized in the whole proximal tubule, but not in the loop of Henle or in the distal portion of the nephron. The cellular components of kidneys from rats that had received large amounts of this mercurial were separated by centrifugation, and the radioactivity was found to be concentrated primarily in the fraction identified with the mitochondria and with a microsomal fraction (23). Whether either site of accumulation relates to diuretic activity, transport of the compound per se, or toxicity is not obvious from these data alone. Sanabria (242) reported that electron microscopic study of cortical and medullary zones of rat kidneys following meralluride injection showed changes in the proximal portions of the tubules, but not in the distal portions. The changes consisted of vacuolation and loss of contrast due to intracytoplasmic edema. There were changes in the brush border, in addition to mitochondrial swelling with vacuolation of the matrix and disappearance of christae. Whether this relates to diuretic activity or toxicity, or both, is not clear. Jones, Lockett & Landon (137) have studied the effect of several organomercurial diuretics and nondiuretics on subcellular organelles and ATPase of the rat kidney. They obtained an endoplasmic reticular fraction and a cell sap fraction by differential centrifugation of whole rat kidneys. They previously reported that the glycolytic activity of

the cell sap fraction was enhanced by addition of the endoplasmic reticulum. This effect on glycolysis was inhibited by the inhibitory effect of the organomercurials on the adenosinetriphosphatase (ATPase) of the reticulum (138). The effect of the diuretics was greater on the Na^+-K^+-activated membrane ATPase than on other ATPase, in agreement with Taylor (274) and Duggan & Noll (84), whereas mercuric chloride inhibited both ATPases much less differentially. When rats were given an organomercurial prior to isolation of the above system, the stimulation of cell sap glycolytic activity by the endoplasmic reticular fraction diminished in a dose-related manner that correlated with diuretic response. Pretreatment with nondiuretic mercurials did not cause a decrease in the reticular ATPase except at high dosages. Thus, they would relate the diuretic activity of the organomercurials they studied to a depression of membrane Na^+-K^+-activated ATPase and, hence, a reduction in cytoplasmic glycolysis. The evidence for involvement of renal membrane ATPase in coupled transport of sodium and potassium (162, 163) is cited to relate these observations to the effect of mercurials on active sodium transport. As Taylor (274) pointed out, such sulfhydryl-reacting substances as iodoacetate and iodoacetamide do not inhibit ATPase, nor do the xanthine or thiazide diuretics. While the evidence for a direct relationship between organomercurial-induced saluresis and inhibition of membrane ATPase is inviting, this relationship seems more apparent than established at this stage of inquiry.

Ethacrynic Acid

The α,β-unsaturated ketone derivatives of aryloxyacetic acids, first synthesized by Schultz et al. (250), are clearly important advances in diuretic therapy. They differ from the thiazides and their functional analogues conceptually, qualitatively, and quantitatively. Their pharmacological characteristics have been described in a series of communications by Baer et al. (10, 11) and by Beyer et al. (36), and their toxicologic assessment has been presented by Peck, McKinney & Zwickey (212) and by Mattis and his associates (193).

These compounds, as characterized by ethacrynic acid, which is 2,3-dichloro-4-(2-methylenebutyryl)-phenoxyacetic acid, form adducts across the unsaturated portion of the methylene structure with many sulfhydryl-containing compounds. Unlike the ability of mercury to react with two SH groups to form a stable cyclic complex (269) (as with the dithiol dimercaprol) which is inactive as a diuretic agent, the corresponding adduct of dimercaprol with ethacrynic acid is either partially active or capable of donating the reactive compound for other adduct formation. Porter (220) has found the cysteine adduct of ethacrynic acid to be its principal urinary metabolite, but, when this compound is administered to dogs intravenously, a diuresis ensues and the free ethacrynic acid as well as the cysteine complex and other metabolites can be isolated from the urine (36). Komorn &

Cafruny (159) demonstrated that protein-bound sulfhydryl groups of renal slices taken from dogs subjected to ethacrynic acid diuresis were diminished, as studied by a technique previously employed to make similar observations on mercurial diuretics (93). This they deduced to support the premise that the mechanism of action of the two classes of compounds was similar (158). Gussin & Cafruny (123) reported also that ethacrynic acid could inhibit the uptake of Hg^{203}-labeled chlormerodrin by renal slices.

Characteristically, ethacrynic acid has a steeper dose-response curve than hydrochlorothiazide, the magnitude of its saluretic effect is severalfold that of the thiazides, its onset of action when administered intravenously or orally is at least as prompt as for the thiazides, and it is at least as potent as meralluride under optimal conditions for organomercurial activity. Like the thiazides, ethacrynic acid is active under conditions of acidosis or alkalosis, but, whereas the thiazides increase the excretion of both bicarbonate and chloride ions during $NaHCO_3$-induced alkalosis, ethacrynic acid increases the excretion of chloride ions only. It does not inhibit carbonic anhydrase *in vitro* (36).

The duration of action of ethacrynic acid is about the same as for chlorothiazide, it is substantially bound to plasma protein (95 per cent), and, like the thiazides, it is secreted by the probenecid-sensitive transport mechanism of the proximal convoluted tubules. Its action is on the kidney, to which organ its effects seem limited except for hepatic secretion.

As mentioned, the mode of action of ethacrynic acid differs qualitatively from that of the sulfonamides. The concomitant administration of maximally effective doses of hydrochlorothiazide and ethacrynic acid gives rise to a saluresis that is severalfold greater than for the thiazide, but in dogs it does not exceed the saluresis which can be induced by the phenoxyacetic acid derivative when administered alone (36). While this observation is subject to alternative interpretations, it suggests that the mode of action of ethacrynic acid may be more fundamental to sodium transport. Duggan & Noll (84) have demonstrated that ethacrynic acid inhibits the Na^+-K^+-dependent adenosinetriphosphatase (ATPase) derived from renal cortex, as do the cardiac glycosides and organomercurials. This is the membrane ATPase identified by Skou (261) with active cation transport. While the affinity of ethacrynic acid for this membrane ATPase is greater than for other tissue ATPase, as is the case for ouabain and the mercurials, its *in vitro* activity is the lowest of these and the plasma concentration required to induce natriuresis is less than its *in vitro* ATPase inhibitory activity. Whereas these imponderables would seem to preclude its effect on this membrane ATPase as being important to its mode of action, nevertheless, the evidence for this association of enzyme inhibition with natriuresis might better be considered presently as inconclusive.

The site of action of ethacrynic acid seems more firmly established. Its ability to inhibit the transport of sodium out of the ascending limb of the loop of Henle distinguishes the compound from organomercurials or the

thiazides. Goldberg et al. (114) showed that in man this drug decreased free water clearance (C_{H_2O}) under conditions of water loading and depressed the back-diffusion of water from the distal portion of the nephron ($T^c_{H_2O}$) under hydropenic conditions. These results were confirmed in man (55, 183) and dogs (36, 87). Ethacrynic acid is capable of abolishing the concentration gradient of sodium from cortex to medulla (36), which gradient Wirz (308) and Gottschalk & Mylle (119) had attributed to the countercurrent multiplier system of the loop of Henle. Baer et al. (8) showed that this gradient was not affected by supramaximal doses of hydrochlorothiazide. This evidence, together with other experiments, has supported the impression that this agent inhibits sodium transport in the proximal and distal portions of the nephron as well as in the loop of Henle (36, 53). The compound does not inhibit the exchange of sodium for potassium in the distal portion of the nephron; hence, as an increased sodium load is presented to this distal system by inhibition of more proximal sodium reabsorption, this Na^+-K^+ exchange is increased and an enhanced excretion of potassium can be demonstrated. The mechanism for this action has been discussed thoroughly in principle by Berliner (25) and is depicted by stop-flow analysis of the effect of ethacrynic acid on sodium and potassium excretion (36).

Cannon, Ames & Laragh (53) reported that ethacrynic acid was a more potent natriuretic and diuretic agent than any known compound and that it proved to be lifesaving in some patients refractory to other diuretic agents. In general, the experience has been that its greater order of activity under general clinical conditions of use has extended the utility of diuretic therapy in patients who had become refractory to other agents (70, 82, 102, 170, 185, 194, 237, 258, 266, 267). Foltz (102) found the dose-response curve for the compound to reach its maximum at about 200 mg/day in man. Kirkendall and his associates (101, 201) reported that in patients effective diuretic doses of ethacrynic acid had no significant effect on systemic arterial pressure, mean right atrial pressure, heart rate, cardiac output, or total systemic vascular resistance, although cardiac output tended to decrease and heart rate appeared to increase. As the dosage was increased, the osmolar clearance increased and the apparent reabsorption of free water ($T^c_{H_2O}$) diminished, as did uric acid clearance, glomerular filtration rate and filtration fraction. Renal blood flow was not altered. Serum uric acid increased and CO_2 tended to rise without changes in sodium, potassium, or chloride. The rise in uric acid blood levels is similar to that induced by the thiazides and for the same reasons. Rosenberg et al. (236) indicated that, like chlorothiazide (78), ethacrynic acid given in large doses intravenously is uricosuric, whereas smaller clinically useful doses induce uric acid retention by inhibiting the active secretion of that metabolite (124). Yü & Gutman (313) reported this generalization to hold for uricosuric agents as well.

The principal shifts in electrolyte balance are the retention of CO_2 secondary to chloruresis, which may be expressed as a metabolic alkalosis,

and the hypokalemia secondary to the kaliuresis induced by the exchange of potassium for sodium in the distal portion of the nephron. The extent of these involvements is dependent on dosage and the nature and severity of the illness (30, 54, 124, 225). Although the CO_2 retention attending ethacrynic acid administration is of greater incidence and consequence than for the thiazides, especially chlorothiazide, its kaliuretic effect does not seem to differ substantially in spite of the greater natriuresis (82, 258). It seems unlikely that it affects carbohydrate metabolism or blood sugar (233). Fisher (100) found no adverse effect of the compound over a period of six months in patients whose insulin requirements had been increased by thiazides.

The toxicity attributable to the drug relates primarily to its effects on electrolyte balance in laboratory animals. Peck, McKinney & Zwickey (212) reported that supramaximally tolerated doses administered to dogs caused dehydration and reduction in plasma sodium, potassium, and chloride ions, with retention of CO_2 and hemoconcentration. The animals died of inanition. Mattis et al. (193) demonstrated that the inanition could be reversed, together with the shifts in electrolyte balance, if the dogs were given saline to drink. Whereupon they began to eat again, they gained weight and thus tolerated an otherwise lethal drug dosage level. Other than the effect on electrolyte balance, which included reduction in plasma sodium, chloride, and potassium, and increased CO_2 and uric acid retention, irritation of the gastrointestinal mucosa was seen. Likewise, gastrointestinal distress and, rarely, diarrhea have been noted by patients. Ethacrynic acid may be employed satisfactorily and safely where diuresis is indicated, and its greater potency extends the range of present therapy.

THIAZIDES

Since the synthesis of chlorothiazide by Novello & Sprague (206) and the initial description of its pharmacologic attributes by Beyer (32), these compounds have become basic therapy in the management of hypertension and the edematous states. Although they are carbonic anhydrase inhibitors and although their discovery was predicated on the thesis that it was theoretically possible to develop a saluretic carbonic anhydrase inhibitor, nevertheless, the known attributes of carbonic anhydrase inhibitors, since the 1937 report by Southworth (265) that sulfanilamide induced an alkaline urine and metabolic acidosis to the discovery of acetazolamide (189, 234), made it generally uninviting at the time to accept this as the mode of action of the thiazides.

It is clear that chlorothiazide acts directly on the kidney, for the injection of the drug into one renal artery of the dog produced a saluretic effect at low dosages in that kidney whether or not it affected the contralateral organ [Lavender & Pullman (168)]. Bartram (18) made a similar observation on the direct effect of a mercurial diuretic on the kidney in 1932. The perfusion of the isolated cat kidney with chlorothiazide

increased the excretion of sodium and water and decreased PAH Tm without affecting renal blood flow (77).

Beyer (32) originally proposed that the primary site of action of chlorothiazide was the proximal portion of the nephron, and this view was supported by stop-flow experiments (148, 287). The most direct evidence that thiazides affect the proximal reabsorption of sodium is the experiments of Beyer et al. (35), which show that the prior administration of probenecid, which prevents the accumulation of chlorothiazide, hydrochlorothiazide, or trichlormethiazide in the proximal segment, can block the natriurctic response to minimally effective dosages of these agents. Increasing the dosage of the thiazide can override the proximal probenecid effect to yield a natriuresis. Employing doses of chlorothiazide 100 times those necessary to induce saluresis, Earley, Kahn & Orloff (88) observed a decrease in the clearance of free water (C_{H_2O}) as the osmolar clearance increased. In hydropenic dogs, the compound did not lower the rate of back-diffusion of water, calculated as $T^c_{H_2O}$, and so they concluded that the compound acted predominantly in the distal convoluted portion of the nephron without affecting sodium transport in the ascending loop of Henle. Still other experiments suggest that there is a chlorothiazide effect on sodium transport in the more distal portion of the nephron (50, 290). Baer, Brooks & Noll (8) found that excessive dosage of hydrochlorothiazide did not influence the concentration gradient of sodium from cortex to medulla, which is consistent with the interpretation that it has no direct effect on sodium transport in the loop of Henle but does not per se prove the point.

Actually, it has seemed that at the lowest effective dosage, the thiazides inhibit the exchange of sodium for hydrogen in the proximal portion of the nephron where they accumulate in the course of their secretion and that, as the dosage is increased to present an adequate concentration to the distal convoluted tubules, the back-diffusion of the compound can inhibit the Na^+-H^+ exchange in that portion of the nephron also (32, 129, 136, 164, 198).

The renotropic characteristics of the thiazides determine in large measure the thousandfold difference in activity of chlorothiazide and cyclopenthiazide [Beyer et al. (35)]. Although all these compounds, including chlorthalidone, are secreted by the probenecid-sensitive system of the proximal portion of the nephron (12, 17, 32, 34, 148, 214, 251), they differ markedly in their lipid solubility, their clearance ratio to glomerular filtration, and the characteristics of their accumulation on the organelles of these cells. Thus, the ether/water partition coefficient most closely parallels the activity of chlorothiazide, hydrochlorothiazide, trichlormethiazide, and cyclopenthiazide as these increase by approximately tenfold increments, and plasma protein binding is greatest for cyclopenthiazide and least for chlorothiazide in this comparison (35). Whereas Essig (92) could not relate systematically the pK_a of several thiazides to their de-

pression of PAH accumulation in renal cortical slices, Duggan (83) showed that, in a comparison of hydrochlorothiazide, trichlormethiazide, and cyclopenthiazide, the first of these was least concentrated on mitochondria and microsomes from rabbit renal cortical cells, and cyclopenthiazide was accumulated to the greatest extent. Whereas hydrochlorothiazide diffused from cortical tubular preparations under conditions of temperature reduction or dinitrophenol addition, the outward diffusion of cyclopenthiazide under these conditions was only discernibly but unimpressively depressed (83). All of these compounds undergo some back-diffusion, presumably in the distal portion of the nephron where they are concentrated in the lumen by the prior outward diffusion of water (35). From a consideration of these several attributes for chlorothiazide, cyclopenthiazide, and the compounds of intermediary activity, it would seem that at lowest dosage these compounds influence sodium reabsorption because of their accumulation at the locus responsible for Na^+-H^+ exchange in the proximal cells of the nephron. Since both glomerular ultrafiltration of a necessarily greater plasma concentration and the amount secreted per unit time are greatest for chlorothiazide and least for cyclopenthiazide, the amount presented to the distal nephron for diffusion into those cells follows in the same order. Since the carbonic anhydrase inhibitory activity of chlorothiazide is about ten times that of cyclopenthiazide, these various factors combine to make the distal effect of chlorothiazide the most easily demonstrable at ordinary dosages (35). Darmady & Mowles (71) reported the autoradiographic localization of hydrochlorothiazide to be in the central portion of the proximal convoluted tubule and throughout the distal convoluted tubules. The physiological economy of chlorthalidone [Stenger, Wirz & Pulver (268)] may relate to substantial enterohepatic circulation as well as its renotropic characteristics (of renal tubular secretion, ultrafiltration, and reabsorption) (35).

Since carbonic anhydrase remains the enzyme most sensitive to thiazide inhibition, the evidence for and against its implication in their mode of action is worth reviewing. Duggan (83) could find no other important effect of these compounds on isolated enzyme systems, including the Na^+-K^+-dependent ATPase of Skou (261), which is identified importantly with active cation transport (300). Davenport & Wilhelmi (72) found carbonic anhydrase to be limited to the cortical portion of the kidney where the proximal and distal convoluted tubules are situated. Hausler (126) provided histochemical evidence for the presence of heavy concentrations of carbonic anhydrase along the luminal border of the proximal portion of the tubules and a lesser concentration along the interstitial border. Evidence for the proximal acidification of urine includes micropuncture data (118, 289), stop-flow experiments (187), and histochemical evidence (204). Walser & Mudge (291) pointed out that the reduction in proximal urinary pH was insufficient to account for the dissociation of HCO_3 to CO_2 and H_2O unless carbonic anhydrase was situated along

this border, as Hausler had found to be the case (126). Clapp, Watson & Berliner (59) reported that acetazolamide inhibited proximal tubular bicarbonate reabsorption. They are of the opinion that their findings are compatible with the Na^+-H^+ exchange process and do indicate the importance of carbonic anhydrase in the proximal tubules, where most of the filtered bicarbonate is reabsorbed (58, 118).

There are at least three bits of information that support, but do not prove, an inhibition of proximal carbonic anhydrase catalysis of the Na^+-H^+ exchange transport and bicarbonate reabsorption by the thiazides. (*a*) At plasma concentrations somewhat less than those required to inhibit carbonic anhydrase *in vitro,* hydrochlorothiazide can accumulate in the proximal cells to inhibit sodium reabsorption unless blocked by probenecid (which neither inhibits carbonic anhydrase nor sodium reabsorption) (35). (*b*) Ross & Cafruny (239) showed that a weakly active thiazide derivative was capable of blocking the saluretic-diuretic effect of hydrochlorothiazide, of reducing urine flow, of increasing urine pH following chlorothiazide, and of having little effect on the response to acetazolamide. We have interpreted these data to indicate an affinity of the weakly active compound EX 4877, which contains a free sulfamyl group, for carbonic anhydrase such that its prior administration blocked the accessibility of the other compounds to the enzyme in the proximal tubules. (*c*) Wirz (309) has indicated that, in micropuncture experiments, hydrochlorothiazide inhibits the proximal reabsorption of bicarbonate along the lines described by Clapp, Watson & Berliner (59) for a similar action of acetazolamide, which likewise is accumulated and secreted by the proximal portion of the tubule in a manner which appears to be qualitatively identical to that of the thiazide (35, 298). Ullrich (284) employed the short-circuit current technique on single tubules of the kidney to show that chlorthalidone diminished the outflux of sodium about 25 per cent in the proximal tubule.

The net difference in anions excreted along with sodium and potassium by the thiazides (predominantly chloride) and acetazolamide (predominantly bicarbonate) has seemed needlessly difficult to reconcile conceptually with a common mode of action. Cyclopenthiazide is about one tenth as active a carbonic anhydrase inhibitor *in vitro* as chlorothiazide, it is some 1000 times more lipid-soluble (ether/water partition coefficient), it is bound on plasma protein to the extent of about 95 per cent (35), and it is difficult to displace once accumulated in the proximal tubules (83). In turn, chlorothiazide is about 1/400 as active a carbonic anhydrase inhibitor as acetazolamide, it is bound to plasma protein to the extent of some 50 per cent of its total plasma concentration whereas acetazolamide is bound to the extent of about 25 per cent or less, and the lipid solubility of acetazolamide is about half that of chlorothiazide. The rate of reabsorption of acetazolamide is about the same as its rate of secretion, whereas it is difficult to demonstrate, except indirectly, a back-diffusion of chloro-

thiazide or cyclopenthiazide. Thus, it is hypothecated that all three compounds inhibit carbonic anhydrase of the proximal convolutions, so that they present to the distal portion of the tubule for reabsorption an increased amount of sodium, potassium, chloride, and bicarbonate ions. If there were no impairment of carbonic anhydrase activity at that distal site, further acidification of the reduced volume of urine along the lines conceived by Pitts and Alexander (216) would lead to reabsorption of some sodium and essentially all the CO_2, leaving an acid or neutral urine containing an increased amount of sodium, chloride, and potassium ions, the latter cation being increased by exchange with sodium in the distal portion of the nephron in response to the increased sodium load brought to that distal site. However, if the carbonic anhydrase-dependent Na^+-H^+ exchange in the distal portion of the tubule is inhibited partially in addition to that situated proximally, it follows that a greater load would be placed on the Na^+-K^+ exchange leading to a more evident kaliuresis, a decreased reabsorption of bicarbonate, and an increase in the back-diffusion of chloride. Because of (*a*) the greater glomerular ultrafiltration of acetazolamide, (*b*) its least lipid solubility as reflected in its proximal transport characteristics, (*c*) the very substantially greater distal reabsorption of this compound, and (*d*) its manifold greater carbonic anhydrase inhibitory activity, it would seem that its effect on the distal portion of the tubules would be the greatest of these compounds at any natriuretic dose (35). At lowest dosages, chlorothiazide has little or no effect on net bicarbonate excretion, but this effect is easily elicited by increasing dosage. In their studies on the mechanism of the diuretic action of chlorothiazide, Pitts et al. (217) concluded that the mechanisms responsible for bicarbonate reabsorption, including the mechanisms for sodium, hydrogen, and potassium exchange, are all affected in a qualitatively similar fashion by chlorothiazide and acetazolamide, presumably as a consequence of their anticarbonic anhydrase effect which is greatest for acetazolamide. Their data showed that large doses of acetazolamide induced a definite increase in chloride excretion, as do the data of Relman et al. (227). It is clear that Pulver, Stenger & Exer (224) interpret their studies on carbonic anhydrase inhibition by thiazides and chlorthalidone to indicate that this is the enzymatic basis for such action.

If the carbonic anhydrase inhibition is to be entertained as the mode of action of these various compounds, several bases for reservation need to be resolved. The first of these was the lack of a direct relationship between carbonic anhydrase inhibition and natriuretic activity, as illustrated by Beyer & Baer (34). More important are the reports that N-substituted sulfamyl thiazides can be saluretic *in vivo,* for Krebs (161) had shown that N-sulfamyl substitution blocked the carbonic anhydrase inhibitory activity of such compounds. Logemann et al. (177, 178) reported that certain N-methyl sulfamyl compounds were diuretic in the rat, but these have been shown to undergo demethylation *in vivo* to give the corresponding

free sulfamyl analogue (181, 310), which in each instance inhibits carbonic anhydrase. Previously, Maren (188) had reported that N^5-alkyl acetazolamides underwent dealkylation with the exception of the N-acetyl derivative. Maren & Wiley (190) have employed the N^7-acetyl analogues of chlorothiazide and hydrochlorothiazide at many times the saluretic intravenous dosage of these compounds to evoke a modest but real saluretic effect typical of the parent compound, with the accumulation of small amounts of chlorothiazide in the urine following large intravenous doses of these compounds. They pointed out that the corresponding N^7-butyryl chlorothiazide undoubtedly dealkylates to chlorothiazide under the conditions of these experiments and that oral administration of the N^7-acetyl chlorothiazide results in extensive dealkylation to chlorothiazide. Whether such cleavage can occur at some locus of accumulation in the proximal cells of the nephron has not been ascertained, but it is certain that the plasma or urinary concentration of such substances gives little insight into their concentration within these cells. Relman et al. (227) administered to dogs high dosages of an N^2-methyl analogue of acetazolamide which was said to have little or no carbonic anhydrase inhibitory activity. They found the acetazolamide analogue to have a natriuretic-diuretic activity identical qualitatively with that of acetazolamide. They concluded that their data were compatible with the view that a significant part of the diuretic action of large doses of acetazolamide resides in an additional property of the drug not related to carbonic anhydrase inhibition (227).

The kaliuretic effect of the thiazides is due to the delivery of additional sodium to the Na^+-K^+ exchange mechanism in the distal portion of the nephron (25). This exchange transport is not inhibited by the thiazides and it does respond compensationally to the increased sodium load. Since this exchange is modulated by aldosterone, it follows that the hyperaldosterone secretion that attends cirrhosis, nephrosis, and certain severe hypertensive states exaggerates still further the kaliuretic response to the drugs (33). The use of enteric-coated formulations of potassium, mostly in combination with thiazides to offset the kaliuretic effect of these agents, has given rise to a rare but discernible incidence of intestinal lesions which may be ulcerative, obstructive, or both and is believed to be attributable to the local release of high concentrations of potassium. This lesion can be reproduced by the administration of enteric forms of potassium with or without thiazides to monkeys, but enteric thiazide formulations without potassium produce no such manifestations of irritation (40, 81). Lawrason et al. (169) have recently summarized the overall aspects of this problem.

Furosemide

4-Chloro-N-(2-furylmethyl)-5-sulfamoylanthranilic acid (furosemide) is a new and unusual diuretic agent which is a carbonic anhydrase in-

hibitor of the same order as sulfanilamide *in vitro*. Kleinfelder (154) reported it to have a steep dose-response curve, like ethacrynic acid (36), and the Na^+/K^+ ratio of excretion of the two compounds appears to be greater than for the thiazides (103), although this does not seem to be a practical consideration (133, 275), as potassium supplementation may be indicated in much the same situations in which hypokalemia is a problem with other diuretics [Schnack (249); Robson et al. (235)]. Its low order of lipid solubility combines with glomerular filtration and renal tubular secretion [Ambrosoli et al. (1); Bergstrom, Hultman & Josephson (24)] to give it a shorter duration of action than ethacrynic acid or the thiazides. It is definitely more potent than the thiazides as far as its maximal effect on salt and water excretion is concerned. Whereas Hook & Williamson (131) found it to be only about 9 per cent more active than hydrochlorothiazide in their studies, it is clear that the compound more closely approximates the order of activity of ethacrynic acid, although the equiactive dose is about twice that of the latter compound (1, 154, 275).

Neither the site of action nor the mode of action of furosemide is clearly defined as yet. It is clear that, administered after chlorothiazide, it causes a greater saluretic response and that hydrochlorothiazide does not enhance the maximal response to furosemide in dogs (131) and in man (235). It does not increase the maximal response to ethacrynic acid or chlormerodrin (131). These observations may relate to differences or similarities in mode of action, site of action, or both. Suki, Rector & Seldin (271) have reported that furosemide increased osmolar clearance and decreased free water clearance. They also report that in hydropenic dogs given antidiuretic hormone and saline venoclysis the free water reabsorption ($T^c_{H_2O}$) decreased (186). These data and those of Buckborn & Anastasakis (45) contribute to the interpretation of an action of the compound in both proximal and distal portions of the nephron (272). Although Hook & Williamson (130) interpreted the fact that furosemide abolished the corticomedullary electroylte concentration gradient to indicate a direct effect of the loop of Henle, these results would require supportive clearance studies to distinguish this site from an effect on the collecting duct.

Presently there is nothing to suggest that the action of furosemide is solely on carbonic anhydrase, or that it affects sulfhydryl-catalyzed enzymes, or that it inhibits membrane ATPase. Karger (142a) has deduced from its effect on the short-circuit electropotential characteristics attending transport of sodium and chloride ions across frog skin *in vitro* that furosemide inhibits chloride rather than sodium transport, and suggested that its renal action may relate more directly to chloride reabsorption than to sodium. The fact that furosemide is active in rats, whereas ethacrynic acid is not (10), may or may not be an adequate basis to distinguish between their modes of action.

The prompt action and substantial potency have made this a useful

agent, as in the management of pulmonary edema following intravenous administration and in some edematous patients unresponsive to other therapy [Schirmeister & Wellman (248); Vorburger (288); Berman & Ebrahimi (27); Heidland, Klutsch & Suzuki (128)]. Its toxicity for laboratory animals relates to its effect on electrolyte and water balance [Muschaweck & Hajdu (200); Thoms, Springman & Wilson (277)], and, although it can produce dehydration and the usual electrolyte changes of hyponatremia, hypokalemia, and hypochloremia induced by excessive saluretic-diuretic therapy, such effects, should they occur, are more attributable to excessive dosage. Like the thiazides and ethacrynic acid, furosemide causes a retention of uric acid [Schaefer (246); Wolfer et al. (311); Hutcheon, Mehta & Leonard (133)] and presumably for the same reasons, as Schirmeister & Wellman (248) have indicated that its intravenous administration usually increases uric acid clearance transiently. Whether or not it will be found to influence glucose metabolism over the course of time, Schaefer (246) found that it did not influence glucose tolerance in his normal or diabetic patients. The rise in blood urea that attends its use is seen with other diuretics as well [Kerr & Robson (145)].

Aldosterone

Recent reviews have been published by Ross (240) and by others (256, 259, 270). The site of action of aldosterone in the kidney is most clearly on the distal convoluted tubule. Micropuncture of the proximal tubules of the *Necturus* failed to reveal an effect of aldosterone on sodium flux at that site [Oben, Flanagan & Khuri (207)], and stop-flow studies in dogs were interpreted to mean that it does not increase sodium reabsorption proximally (286). Since the relevance of the negative *Necturus* experiments is uncertain and the dog is remarkably insensitive even to large amounts of aldosterone administered into the renal artery (16), it is reassuring that other studies including man are consistent with the impression that the proximal reabsorption of sodium is not altered by the hormone (115, 264). It is hard to distinguish whether such a weakly effective compound actually acts on the loop of Henle directly or merely appears to as its influence on the more distal segment extends to the collecting ducts as well as to the distal convoluted tubules. Thus, the aldosterone-increased concentrating ability of the human kidney on which Crabbé (65) based his interpretation that it increased the activity of the loop of Henle is at variance with the findings of others whose results led them to another interpretation, as discussed below. Although Crabbé & Nichols (67) were unable to demonstrate an effect of the hormone on the uptake of sodium incubated with rat renal cortical slices *in vitro,* Kessler et al. (146) were able to show that, when aldosterone was administered to normal rats, the kidneys subsequently removed and analyzed for sodium had a higher content of this cation in the cortex, medulla, and papilla than was present in the control animals (146). While a stimulation of sodium re-

absorption by the loop of Henle could account for the high medullary and papillary sodium content, these results could be attributed also to an analogous effect on the collecting duct, which was the interpretation Platts (218) placed on similar experiments. Vander et al. (286) concluded from their stop-flow experiments in dogs that aldosterone acted on the more distal portion of the nephron. The data of Sonnenblick et al. (264) and of Yanis (312) pertaining to the reabsorption of sodium were interpreted in a similar manner, the latter author placing the site of action in the terminal portion of the distal convoluted tubule and in the collecting duct.

That aldosterone exerts a modulating or facilitatory effect on the exchange of sodium for potassium is generally supported by several approaches to the problem, but whether this is its only renotropic effect or how it works is less certain. Feldman et al. (98, 99) concluded from their studies that such changes as they observed to attend the effect of aldosterone on rat kidney enzymes were inhibitory and probably nonspecific and that quite likely it required an intact cell system to exert its effect. Nelson & Cornatzer (203) reported that aldosterone stimulated the synthesis of phosphatidylcholine of kidney mitochondria and nuclei and phosphatidylethanolamine of mitochondria on the basis of inorganic P^{32} incorporation, and Davidson, Devenuto & Westphal (73) indicated that the hormone was accumulated and bound by liver and kidney subcellular fractions and cell membranes. Edelman and his associates (91, 221) have contributed importantly to insight into the actions of aldosterone. They reported that the hormone stimulated nuclear DNA-dependent RNA synthesis. Prior incubation of their toad bladder system with actinomycin or panomycin inhibited its effect on nuclear or ribosomal RNA protein synthesis and abolished the enhancement of sodium transport by aldosterone. The hormone increased the incorporation of C^{14}-valine into protein (257) and uridine into ribonucleic acid (90). RNA synthesis preceded the delayed effect of the hormone on sodium transport [Porter, Bogoroch & Edelman (221)], and glucose or pyruvate was required for the aldosterone-induced increase in high energy phosphate synthesis which, together with sodium transport could be inhibited by oligomycin. The sodium transport paralleled the synthesis of a phosphorylated intermediate which reacted with ADP to give ATP, but it was felt that ATP was not the source of energy for sodium transport [Davis et al. (74)]. Perhaps this relationship of prior effect on synthesis to apparent effect on sodium transport accounts for the delay in onset of action observed for aldosterone in laboratory animals and man [Ross et al. (241)]. The effect of aldosterone on sodium transport across the toad bladder is delayed an hour or so, in spite of the fact that tritiated aldosterone can be found in such bladder walls in substantial concentration within 15 minutes of its administration (64, 66). If it should be that aldosterone acts by facilitating the action of Na^+-K^+-dependent membrane ATPase, as identified with the fundamental Na^+-K^+ pump mechanism, then its site of action in the more distal portion of the nephron may relate more to the localization

of effective concentrations of the hormone at that site. This action of aldosterone could account, perhaps, for the increased reabsorption of sodium at that distal site under hormonal influence, but it does not seem to relate to or account for the substantial distal secretion of potassium or the effect of triamterene on Na^+-K^+ exchange at that site in the presence or absence of aldosterone.

Aldosterone Antagonists

Aldosterone antagonists of the nature of steroidal 17-spirolactones have been described chemically by Cella, Brown & Burtner (57) and by Kagawa, Cella & Van Arman (141), who proposed that these were truly antagonists since they did not act in adrenalectomized animals unless aldosterone or another mineralocorticoid was administered prior to the spirolactone. Liddle (175) supported this view when he found the antagonists to produce the same effects as reduced aldosterone synthesis due to the administration of SU-4885, even though they tend to increase rather than decrease aldosterone secretion, indirectly. These drugs reverse all known actions of aldosterone on the kidney (176). Kagawa et al. (140) have recently described SC-14266 as a water-soluble steroid which has the mineralocorticoid antagonist attributes of spironolactone. Coppage & Liddle (63) reviewed the mode of action and usefulness of these compounds, as did Ross (240). The clinical literature continues to support their utility, especially when coadministered with the thiazides. In this combined therapy, spironolactone antagonizes usefully the kaliuretic effect of the thiazide by its antialdosterone effect. The thiazides, in turn, provided the needed saluretic potency that is only marginally provided by the spironolactone. Kagawa & Drill (142) documented this basis for the interaction of spironolactone and hydrochlorothiazide in rats.

By virtue of its aldosterone antagonism, the renal site of action of these compounds is limited to the distal convoluted tubules and collecting ducts, as discussed for aldosterone, but there is little or no insight into the nature of the antagonism.

Triamterene

Triamterene is not an aldosterone antagonist, but it does inhibit the exchange of sodium for potassium in the distal portion of the nephron. Wiebelhaus et al. (304) first reported that this triaminophenylpteridine was capable of blocking the Na^+-K^+ exchange mechanism in adrenalectomized rats and dogs, and this has been confirmed by Baba, Tudhope & Wilson (4), thus distinguishing it qualitatively from the spirolactones that are active in such animals only when they have been treated with aldosterone or a mineralocorticoid such as 9α-fluorohydrocortisone. Characteristically, the compound moderately increases the excretion of sodium and bicarbonate and chloride to a lesser degree. It reduces potassium and ammonia excretion (69, 113, 165) and potentiates the action of the thiazides while reducing their kaliuretic

effect (56). The duration of action of a clinical dose is about 16 hours (20), and some 20 to 30 per cent of the oral dose can be recovered in the urine within 24 hours (6, 7, 167), although it may continue to be excreted for five to seven days (107).

Ball & Greene (15) could find no evidence for a proximal renal tubular effect of triamterene. Their stop-flow studies in dogs pointed to an effect on the distal site on Na^+-K^+ exchange. This impression was supported by stop-flow experiments on pigs [Nielson & Lassen (205)] and by the predominant localization of tritiated triamterene in the distal tubules in the experiments of Baba, Tudhope & Wilson (5), although they did note some radioactivity proximally as well. Perhaps the proximal radioactivity relates more to the evidence that the compound is secreted by the tubules [Lassen et al. (167); Nielsen & Lassen (205)]. How the drug influences cation transport is not clear. Baba, Tudhope & Wilson (5) have reported that it inhibited the active transport of sodium by frog skin preparations, but only when applied to the external surface. Other reports indicate it is inhibitory when applied to frog skin preparations *in vitro* [Bronstein et al. (44)] and facilitatory in intact frogs [Maetz, Jard & Morel (184)]. Kagawa, Baran & Krol (139) have reported that triamterene inhibited the strong kaliuretic effect of ouabain administered to adrenalectomized rats. Spironolactone did not. That very fundamental differences exist between the aminopteridines and aldosterone antagonists finds clinical expression in the reports that an increased diuresis was noted when triamterene was administered to patients who were receiving spironolactone (56, 282). Even so, the natriuretic-diuretic potency of these present compounds that influence the Na^+-K^+ exchange mechanisms is substantially less than for the thiazide and phenoxyacetic acid saluretic agents to which they are useful adjunctive therapy.

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