RENAL PHARMACOLOGY[1,2]

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In the preparation of the first review of an annual series, the authors have a unique opportunity. A consideration of only material published in the last year or two would hardly suffice as an adequate background for the reviews to follow; it therefore seems essential to include a wider selection of work to define more adequately the present status of the field. At the same time, freed of the necessity of providing a more or less complete bibliography of material related to the assigned subject for the immediately preceding period, the authors have more freedom in the choice of work to be included and in the presentation of selected areas of study.

Pharmacological interest in the kidney is directed largely along two lines —the effect of drugs on the transport mechanisms of the renal tubules and the effect of drugs upon the renal circulation and glomerular activity. This review will deal primarily with the renal transport mechanisms for electrolytes and water and the action upon them of several groups of diuretics. Limitations of space have required that several subjects of considerable current interest (e.g., the actions of hormones on renal function, uricosuric agents) be omitted except where directly pertinent to other material.

The regulation of salt and water excretion is, from the point of view of the amounts of substance involved, far and away the most important function of the renal tubules. In addition, it has been the function most effectively subjected to pharmacologic modification for therapeutic purposes. It is pertinent to a consideration of substances which affect these transport mechanisms to review current concepts of the site magnitude, and nature of the processes involved.

Functional Organization of the Kidney

Proximal tubule.—Recent micropuncture studies have, in general, confirmed earlier inferences (1, 2, 3) concerning the extent of sodium, chloride, and water reabsorption in the proximal convoluted tubule and concerning the primacy of active sodium transport in this process. In confirmation of the earlier studies of Walker *et al.* (2), Lassiter, Gottschalk & Mylle (4) have found that in the rat some 65 per cent of the glomerular filtrate is reabsorbed in the convoluted portion of the proximal tubule. Extrapolation to the end of the proximal tubule yields a figure approximating the 80

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] Abbreviations used in this chapter include PAH (*p*-aminohippurate).

per cent estimated earlier (2). The finding that in amphibia (5) and in mammals under the influence of antidiuretic hormone (2, 6, 7) the fluid in the proximal tubule is always isotonic with plasma has been extended to the rat in water diuresis (6, 7). This suggests, as has long been assumed, that the proximal tubule has a high water permeability and that this high permeability is not influenced by antidiuretic hormone. This permeability has been measured directly in the amphibian, *Necturus,* by Whittembury and his associates (8), who have, in addition, demonstrated the entirely passive nature of water movement in this segment, by showing that net water movement out of the tubule lumen is entirely dependent upon net solute movement (9). (In fact, under the conditions of their study which involved instillation of solutions of defined composition into the tubule, it was possible to produce net solute movement, and hence net water movement, into the lumen, in the direction counter to the normal net flux of solute and water.)

The early view emphasized by Bayliss (10) that the movement of fluid and solute out of the proximal tubule might result from the osmotic force provided by the plasma protein in peritubular capillaries has undergone spirited, if short-lived, revival in the last few years (11, 12). Evidence opposed to this possibility has developed rapidly. Whittembury and his coworkers (8) have shown that the inclusion of plasma albumin at concentrations exceeding that in peritubular blood has a negligible effect on the movement of solute and water out of the tubule of *Necturus.* Furthermore, from their measurements of the permeability of the proximal tubule to water, they have been able to calculate the rate of water movement which might be produced by an osmotic pressure of the magnitude attributable to the plasma proteins, and this proves to be a negligible fraction of that actually observed (8). On the other hand, there is abundant direct evidence supporting earlier inferences (13, 14) that the movement of sodium out of the proximal tubule involves transport against a gradient of electrochemical potential and is thus, by definition, "active." The lumen has been found by Solomon (15) in the rat and by Giebisch (16) and Schatzmann *et al.* (17) in *Necturus* to be electrically negative with respect to the interstitial space of the kidney by approximately 20 mv. The movement of sodium from lumen to blood is thus against an electrochemical gradient, even if, as is usually the case, there is virtually no difference between the chemical sodium concentration of the fluid in the lumen and that in the blood. However, when the tubule contents include a considerable amount of solute to which the tubules are impermeable (e.g., mannitol) the reabsorption of sodium reduces the concentration of sodium in the lumen producing a gradient of chemical concentration as well as electrical potential. This has been shown to be the case both in the rat (18) and in *Necturus* (9). It should be noted, however, that the concentration gradient against which sodium can be transported is limited, so that in *Necturus,* in which the studies are more complete, net outward movement ceases when the con-

centration of sodium in the tubule has been reduced to about 60 per cent of that in the plasma (9). This is presumably attributable to the high passive permeability of the tubule to sodium, the diffusion leak of sodium along its electrochemical potential gradient just balancing the capacity of the tubule to transport it outward.

As for the movement of chloride, the direction of the electrical gradient and the high permeability of the proximal tubule to this anion are sufficient to account for its reabsorption from the tubule as a passive process (19, 20). However, the data of Giebisch (20) indicate that the ratio of outflux to influx as measured with tracers is not as large as would be expected for a simple process of diffusion as predicted from the flux ratio equation of Ussing (21). This type of departure from the flux ratio equation is usually taken to indicate the involvement of exchange diffusion (22, 23), and the latter phenomenon is usually considered to suggest the participation of a carrier in the ion movement. It may be, therefore, that in its movement across the tubule cell, chloride may combine with some cell component so that its passage is not entirely in the free state, even though it is energetically downhill.

As in the case of such transport processes generally, virtually nothing is known about the details of the active process by which sodium is moved from lumen to interstitial space. In the elucidation of such mechanisms, their intimate nature, energy sources and substrates, lies the key to the rational development of agents for their modification. To date, the development of drugs modifying renal electrolyte transport has been entirely empirical.

Loop of Henle.—Considerable recent interest has centered upon the loop of Henle because of its involvement in the formation of hypertonic urine. Although it seems clear that, as first proposed by Wirz, Hargitay & Kuhn (24), the loop acts as a countercurrent multiplier and that the primary active process involved is transport of sodium from lumen to medullary interstitial fluid, considerable question remains as to the details of the process. It is clear that in animals excreting hypertonic urine the fluid flowing through the loop of Henle becomes concentrated as it flows down the descending limb, reaching, at the tip of the loop, a concentration not measurably different from that of the final urine (7, 24) and then becoming diluted even more rapidly as it flows back up the ascending limb to the cortex (6, 7). It seems most likely that the rising concentration in the descending limb is due primarily to loss of water to the hypertonic surroundings (24, 25), although, at present, it can not be rigorously excluded that there is not some uptake of sodium chloride in addition to the net gain of urea which has been shown to occur (4). Thus, the descending limb appears, at least in antidiuresis, to share the permeability properties of the proximal tubule. Whether, in the absence of antidiuretic hormone, it may become considerably less permeable to water as does the distal tubule remains to be determined. The dilution which occurs in the ascending limb is

clearly a result of the removal of sodium and chloride (not to the addition of water) since the fluid arrives in the distal tubule, not only dilute (6, 7) but reduced in volume below that at which it entered the loop (4). The characteristics of the process that moves sodium chloride out of the ascending limb are unknown. It is generally assumed to involve the active transport of sodium and the secondary movement of chloride, but this is based on analogy rather than evidence. The countercurrent multiplier effect, which provides a high salt concentration within the lumen at the point at which the interstitial concentration is also high and a lower surrounding concentration when the internal concentration has been reduced, keeps small the concentration gradient against which sodium chloride is transported so that it is not known there is a limitation similar to that found in the proximal tubule. It is important to note that the salt transported out of the ascending limb plays a central role in both the process by which the urine is concentrated and that by which it is diluted. It is the salt moved from the loop and deposited in medullary interstitium which maintains the high osmotic pressure around the collecting ducts (26). At the same time a very considerable dilution occurs which, if maintained, is sufficient to account for a major fraction of the solute-free water excreted in water diuresis.

Distal convoluted tubule.—The generally held view of the role of the distal convoluted tubule in water and electrolyte excretion has been somewhat blurred by the demonstration of considerable transport activity in the collecting ducts in the hamster (27, 28). As a result, there is some uncertainty concerning the extent to which activities formerly attributed to the "distal tubule" really are located in the distal convoluted tubule or the collecting system. In any case, it appears that both segments share one important property, namely, a low permeability to water in the absence of pituitary antidiuretic hormore (vasopressin) and a high permeability in its presence (6, 7, 29). In the presence of antidiuretic hormone, the excess of water over solute present in the contents of the first part of the distal convoluted tubule is, at normal rates of flow, more or less rapidly dissipated (6, 7) as it progresses down the tubule, and the fluid remains close to osmotic equilibrium with the peritubular fluid thereafter. In water diuresis, on the other hand, the permeability of the distal system is sufficiently low to maintain the dilute character of the urine despite the much higher osmotic pressure of the peritubular fluids.

Certain differences in sodium transport are apparent between proximal convoluted tubule and distal system. The most striking of these is the ability of the distal system to reduce the salt concentration virtually to zero as compared with the limited concentration gradient which can be established in the proximal tubule. In addition, a fraction of the removal of sodium in the distal system involves replacement of the sodium with potassium, hydrogen, and ammonium ions and is thus not accompanied by an equivalent abstraction of anion (30, 31). On the other hand, it is not certain to what extent these differences are attributable specifically to dif-

ferences in the sodium transport mechanism itself or to differences in such parameters as the passive permeability of the tubule cells to sodium, potassium, chloride, and water, and to the modification which has already occurred in the composition of the fluid in the tubule lumen by the time it reaches the more distal portions of the nephron.

Measurements of the electrical potential gradient across the distal convoluted tubule are less numerous than those which have been made in proximal convolutions. Contrary to the early findings of Wilbrandt (32), all recent determinations have indicated that the lumen is negative with respect to the interstitial fluid (15, 16). In addition, the magnitude of the electrical potential gradient has been greater in distal than in proximal tubules. Thus the direction of the potential is favorable to the loss of chloride from the lumen by diffusion and is sufficient to produce an appreciable gradient of chemical concentration. However, the production of the frequently observed urinary concentrations one-hundredth that in plasma would require potential gradients of the order of 120 mv. or more. This is approximately twice as great as any which have been measured to date. It is clear that the conditions under which the few distal tubule potentials have been measured are not necessarily those under which maximum chloride reabsorption occurs; nevertheless, it can not be accepted as established that chloride reabsorption in this segment does not require an active transport mechanism.

Collecting duct.—The recent studies of Ullrich and his associates (27, 28) and of Hierholzer (33) have established that, in the hamster, many of the electrolyte transport processes attributed to the "distal tubule" are present in the collecting ducts as well as, presumably, the distal convoluted tubule. The extent to which a similar situation exists in other species remains to be determined, although the studies of Jaenike & Berliner (34) suggest that there may be removal of sodium and chloride and addition of potassium in this segment in the dog. The reviewers do not share the view of Ullrich that the salt transported out of the collecting duct contributes to the process of forming hypertonic urine, but they believe that it must contribute to the over-all regulation of sodium excretion as well as that of potassium, acid, and ammonia and to the dilution of the urine in water diuresis. A contribution of the collecting system to the dilution of urine, at least in rodents, is apparent from the studies of Wirz (6) and Gottschalk (7).

The studies involving fluid collection from the collecting ducts of the hamster did not include measurements of chloride concentration. Therefore, although it is established that sodium reabsorption occurs in this segment, it is not clear whether this is entirely attributable to the demonstrated cation exchanges or whether there is also some removal of chloride. The studies of Jaenike & Berliner (34) suggest that chloride is reabsorbed from the collecting system of the dog kidney. If the latter process occurs, it will be difficult, indeed, to explain it entirely as the passive result of an electrical potential gradient because (*a*) the very high chloride concentra-

tion of medullary interstitium would add to the chemical gradient and thus impose the necessity of some 40 to 50 mv. beyond that required for similar movement in the distal convoluted tubule (see above) and (*b*) the large caliber of the collecting ducts and the short distance to the pelvis would make it difficult to maintain such a high potential in the face of the considerable shunt represented by the conductance of the fluid in the collecting ducts. These considerations again emphasize the necessity of retaining the possibility of active chloride transport in any view of renal transport processes and their modification by drugs.

Potassium.—The specific contribution of various tubule segments to the modification of potassium excretion is by no means as clear as is the case for sodium, chloride, and water. Considerable evidence has accumulated in support of the view that a major part of the filtered potassium is reabsorbed before most of the potassium destined for excretion is secreted into the tubule fluid (35, 36, 37). It is clear that all or virtually all of the filtered potassium can be reabsorbed since urine almost free of potassium can be produced. Unless it should be unexpectedly shown that there is at times complete reversal of the electrical gradient, the excretion of a urine with a potassium concentration lower than that in plasma requires active transport of the potassium out of the lumen. There is, in addition, considerable experimental support for the view that, even under conditions in which large amounts of potassium are excreted, all or very nearly all of the filtered potassium is reabsorbed and that which appears in the urine is derived from the secretory process (36). However, the site of the reabsorption is uncertain. Earlier micropuncture studies suggesting a lowering of potassium concentration in the proximal tubule in both *Necturus* and rat (38, 39) have not been confirmed in more recent work (40), and the latest observations of Oken & Solomon (41) indicate that in *Necturus* the concentration in the lumen of the proximal tubule is higher than in plasma and close to the value required by the electrical potential with potassium at electrochemical equilibrium—that is, with the electrical gradient equal and opposite to the chemical concentration gradient. If the same is true in the mammal, this would require that the active reabsorptive process be located at a more distal site. However, some caution is required in this interpretation since there have been no studies of the gross behavior of potassium excretion in *Necturus* to indicate that it is similar to that in mammals. In stop-flow studies in the dog, the lowest potassium concentrations are found in those "early distal" samples which also contain the lowest sodium concentrations (42). This has been interpreted by Sullivan *et al.* (42) as indicating that these samples delineate the site of maximum potassium reabsorption. This conclusion is certainly not warranted by the evidence for reasons which have previously been presented *in extenso* (43, 44). In essence, one can not distinguish whether the potassium concentration of these samples is low because of potassium reabsorption at that site or because, having been rendered virtually sodium-free, these samples can not take up potassium in ex-

change for sodium as they subsequently flow past the site of potassium secretion. It may be concluded that the site of potassium reabsorption is not distal to the locus proposed by Sullivan *et al.* but could be situated there or at any point proximal to it (44). These considerations become important in the interpretation of stop-flow studies of pharmacologic agents (see below).

Some confusion concerning the role of the distal convoluted tubule versus that of the collecting duct in the secretion of potassium has arisen from the observations of Hilger *et al.* (45) suggesting potassium secretion in the collecting system in the hamster. Although later, more extensive observations by the same investigators (27) failed to establish the occurrence of such secretion under the conditions of study, Hierholzer (33) has demonstrated that potassium secretion does take place in the collecting ducts of potassium-loaded hamsters after acetazoleamide injection. The experiments of Jaenike & Berliner (34) suggest that there is potassium secretion in the collecting system of the dog as well, but indicate that the process extends well back into the distal tubules. The latter conclusion can be reached a priori, in any case, because the amounts of potassium that can be secreted exceed considerably the amount of potassium in the very low blood flow to the renal medulla (44).

The mechanism of potassium secretion clearly involves exchange for sodium ion. This conclusion has received additional support in the last few years (36, 46). It has not been established that the process is active as far as the movement of potassium is concerned, i.e., the electrical gradient with lumen negative to interstitial fluid may be sufficient to account for the accumulation of potassium in the urine. However, as in the case of chloride, the electrical potential required to account for the observed concentration is greater than any which has yet been shown to exist. Whether or not the potassium accumulation is attributed to an active process may prove to be a semantic question in view of the indications that in other analogous situations the active transport of sodium, presumed to be the initiating process in establishment of the electrical potential gradient, involves specific exchange of sodium for potassium (47, 48).

Bicarbonate, urinary acidification, and ammonia excretion.—Measurements of the pH of fluid collected from the proximal convoluted tubule of the rat (49) have confirmed the inferences of Walker *et al.* (2) that the bicarbonate concentration of fluid in the proximal tubule of this species is lower than that in plasma. Although it has generally been accepted in recent years that bicarbonate reabsorption in the proximal tubule, now indicated as being of the order of 90 per cent or more of the filtered bicarbonate, is attributable to exchange of hydrogen ion for reabsorbed sodium, some of the consequences of this assumption have been pointed out for the first time by Walser & Mudge (50). The hypothesis implies the combination of the secreted hydrogen ion with bicarbonate to form carbonic acid which in turn is split to CO_2 and water, the CO_2 escaping by diffusion. This requires, then, that carbonic acid be broken down at a rate equal to that at which

bicarbonate is reabsorbed. In order to achieve this rate, (*a*) there must be accumulation of carbonic acid to a concentration providing the indicated rate of breakdown by the uncatalyzed reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$ (the required carbonic acid concentration would produce a pH approximately one unit lower than that achieved when the above reaction has come to equilibrium) or (*b*) the reaction in the lumen must be catalyzed (one would presume by carbonic anhydrase on the cell surfaces since it is clearly not present in the urine). The technique used for the pH measurements which have so far been made does not exclude the possibility of a pH *in situ* lower than that observed after isolation of the sample for analysis (49, 51), but it seems rather unlikely that this is the case. The remaining alternatives would then be (*a*) a complete revision of our views of the mechanism of bicarbonate reabsorption to one which does not involve formation of carbonic acid in the lumen or (*b*) a new role for carbonic anhydrase in the process—i.e., the catalysis of carbonic acid breakdown in the lumen.

The localization of urinary acidification and ammonia excretion in the distal segments of the mammalian kidney, assumed since the observations of Montgomery & Pierce (51) and Walker (52) in amphibia, has been confirmed on the basis of stop-flow studies (42, 53, 54). An appreciable part of the ultimate lowering of pH apparently may occur in the collecting system in the rat (49) and the hamster (55). Secretion of ammonia also occurs in the collecting ducts of the hamster (28), but it is unlikely that this is the major site of secretion since the supply of precursors of ammonia to this segment is so limited by the low level of medullary blood flow (44). Furthermore, in the rat, the rate of excretion of ammonia is very well correlated with adaptive changes in the glutaminase activity of the cortex while these adaptive changes are absent in medulla and papilla (56, 57).

Transport and excretion of organic acids and bases.—The renal transport mechanism which has probably been more extensively studied than any other is that responsible for the secretion into the urine of a wide variety of organic acids, most familiarly *p*-aminohippurate (PAH), diodrast, and phenol red. The similar but separate mechanism which transports a number of organic bases into the urine has been more difficult to study because of the generally high physiologic activity of most of its substrates. Taggart (58) and Sperber (59) have reviewed the process by which organic anions are transported while Peters (60) has reviewed the transport of organic bases.

The most important development in this field has been the clarification of the interrelationship between active transport and passive diffusion in the excretion of a number of organic compounds in both the acidic and basic series. It has been known for some years that the rate of excretion of a number of weak acids and bases is dependent upon the pH of the urine, the excretion of weak acids increasing, and the excretion of bases decreasing as urine pH increases (61). This has been attributed to the fact that cell

membranes are generally highly permeable to the lipid-soluble un-ionized species but very much less so to the ions (62), a phenomenon which has been designated as nonionic diffusion (63). Since permeation of the tubule is limited almost exclusively to the un-ionized form, diffusion equilibrium is reached only when the un-ionized form is present in equal concentration on both sides of the membrane; the total concentration of the weak acid or base required to provide a given concentration of the undissociated species is, of course, a fuction of pH. Although there can be little question of the general validity of this interpretation, it has become apparent that in the excretion of at least a number of these weak acids and bases more is involved than non-ionic diffusion alone. It was found by Gutman *et al.* (64) and by Schachter & Manis (65) that the excretion of salicylate, a substance showing the relationship between urine pH and excretion typical of nonionic diffusion, was markedly depressed by probenecid, a well-known inhibitor of the active transport mechanism responsible for the secretion of PAH and other organic anions. This phenomenon has been explored extensively by Weiner, Washington & Mudge (66) who present convincing evidence that salicylate is secreted in the proximal tubule by the same mechanism that effects the transport of PAH and that it is subsequently variably reabsorbed by passive nonionic diffusion from the distal nephron at a rate inversely related to the pH of the urine. They show that salicylate secretion is inhibited not only by probenecid but by PAH, acetazoleamide, and benzmalecene as well. The same group has explored this type of phenomenon further and has found a wide variety of organic acids to exhibit similar behavior (67). Probenecid is perhaps the most striking example since it has long been considered the typical "refractory substrate" inhibitor—that is, a substance with a high affinity for the transport mechanism but not itself subject to transport (68). Although it has an extremely low clearance under usual conditions of acid or neutral urine, probenecid is excreted at rates far in excess of that at which it is filtered when the urine is rendered alkaline (67); furthermore, its excretion is depressed by PAH. As Weiner *et al.* point out (67) these findings introduce new complications into the already confusing question as to the structural requirements for active secretion of organic acids. They further indicate that if such secretion is to result in a high rate of urinary excretion, the organic acid must have a low lipid solubility or the urine a very high pH.

Meanwhile it has become apparent that similar considerations apply to the excretion of weak bases. Volle, Green & Peters have found that quinine, quinacrine, and mecamylamine inhibit the secretion of *N*-methylnicotinamide in the chicken (69), although the first three substances generally have rather low clearances and are among those which, in the dog, have clearances which vary greatly with urine pH (61, 70, 71). Torretti *et al.* report that in the dog there is evidence of active secretion of quinine and that nonionic diffusion is concerned chiefly with its loss from rather than uptake

into the urine (72). It thus appears that among the bases as well as the acids the structural requirements for active transport are extremely nonspecific.

Chlorothiazide and Related Compounds

Chlorothiazide, synthesized by Novello & Sprague (73) and introduced as a diuretic by Beyer and his associates (74), has become the prototype of an extensive and still expanding series of heterocyclic sulfonamides with diuretic properties. These compounds have considerable pharmacologic and therapeutic interest not only because of their intrinsic activity but also because they are highly effective on oral administration. The original compound was the by-product of an attempt to produce a carbonic anhydrase inhibitor of greater potency by the inclusion of two functional sulfonamide groups. The benzothiadiazine compound showed an unexpected capacity to produce enhancement of chloride excretion in addition to those actions usually associated with carbonic anhydrase inhibition. Although the capacity of chlorothiazide to inhibit carbonic anhydrase was at first considered the basis of its chloruretic action (74), it has become increasingly clear that these are separate properties. Most of the more recently introduced compounds of more or less related chemical structure have considerably enhanced chloruretic activity but diminished potency as carbonic anhydrase inhibitors.[3] It has, in fact, been reported by Logemann *et al.* (75) that several similar compounds substituted on the sulfonamide nitrogen, and therefore devoid of carbonic anhydrase inhibitory activity, retain the capacity to produce diuresis. The relationship between structure and activity has not been established; nearly all of those which have been so far introduced are derivatives of benzothiadiazine, but there are exceptions (76) which indicate that this ring structure is not itself essential.

Administered in fairly large doses, chlorothiazide increases the excretion of sodium, chloride, potassium, and bicarbonate (74, 77). Maximal effects with approximately 10 per cent of the filtered sodium and chloride excreted in the urine are produced in the dog at doses of approximately 10 mg./kg. (77). When maximally effective doses of a mercurial diuretic are superimposed upon the chlorothiazide diuresis, there is a marked further increase in sodium and chloride excretion. When the order of drug administration is reversed, similar additive effects are produced. From this Pitts and his associates (77) infer that the effects are independently exerted on separate systems yielding energy for the mechanism which effects the tubular reabsorption of sodium.

On the other hand, there was no further increase in the excretion of potassium and bicarbonate when chloromerodrin was administered to a dog undergoing chlorothiazide diuresis, but the excretion of potassium is mark-

[3] Because of space limitations and because there have been no major developments in the study of the renal effects of carbonic anhydrase inhibitors since the subject was last reviewed (82), this group of agents is not dealt with in this review.

edly inhibited (77) indicating that the enhanced potassium excretion is attributable to enhanced secretion rather than inhibited reabsorption (78). The effects of chlorothiazide on the excretion of bicarbonate and potassium are entirely characteristic of the effects of carbonic anhydrase inhibitors, and there was no enhancement of these effects when chlorothiazide was given to animals pretreated with the more powerful carbonic anhydrase inhibitor, acetazoleamide. That these effects are, indeed, attributable to the carbonic anhydrase inhibition produced is further indicated by the facts that (*a*) smaller doses of chlorothiazide may produce the enhanced excretion of sodium chloride without significant effect on urine pH or potassium excretion (74), (*b*) derivatives of chlorothiazide possessing higher ratios of chloruretic to carbonic anhydrase inhibitory activity yield high rates of sodium and chloride excretion without modifying bicarbonate excretion and with only those increases in potassium excretion to be expected, for reasons discussed below, from any diuretic agent (79, 80).

The major diuretic effects of chlorothiazide are not modified by acidosis or alkalosis (74). This is a significant departure from the situation with organic mercurials, the effects of which are markedly suppressed by administration of alkali (see below). On the other hand, failure of metabolic acidosis to suppress the action of chlorothiazide is a departure from the behavior of drugs owing their action entirely to the capacity to inhibit carbonic anhydrase (81, 82), although the absence of enhanced bicarbonate excretion when chlorothiazide was given to acidotic animals (74) may be interpreted as suppression of this facet of chlorothiazide action.

Since the production of potassium depletion has been one of the few limitations in the therapeutic use of chlorothiazide and its congeners, a more detailed consideration of the effects of these agents on renal tubular transport and excretion of potassium is warranted. That carbonic anhydrase inhibitors increase potassium excretion and that this effect is specifically attributable to enhanced secretion of potassium is well established (54, 78). Since significant renal carbonic anhydrase inhibition is produced with most therapeutic doses of chlorothiazide, marked increases of potassium excretion are to be expected. However, since many of the more potent derivatives are diuretic at doses which yield negligible carbonic anhydrase inhibition, the effect on potassium excretion is predictably and demonstrably much smaller (81, 82). However, all diuretics share the property of increasing the excretion of potassium, even though they have no specific effect on potassium transport or despite the fact that, as is the case with mercurial diuretics, they may have the specific capacity to inhibit the secretion of potassium by the renal tubules. This property derives from the fact that, to the extent that they are effective, they enhance the delivery of sodium salts to the site of sodium-potassium exchange in the more distal portions of the nephron and thus permit the more efficient utilization of the capacity to secrete potassium (83, 84). Although stop-flow studies of the effects of chlorothiazide have been interpreted as indicating inhibition of potassium reabsorption (85), these conclusions are

not valid since the method does not distinguish diminished reabsorption from enhanced secretion as has been previously pointed out (44).

A further factor tending to potassium depletion when benzothiazide diuretics are used relates to the increased capacity to reabsorb sodium and secrete potassium, which is a feature of those individuals with disorders leading to the formation of edema and in individuals depleted of sodium. Many of those to whom benzothiazide diuretics are administered fall in one or the other of these categories. It is to be expected that in such individuals loss of potassium will be considerably greater than when a diuretic is administered for the first time to a normal individual. Indeed, a progressive increase in potassium excretion can be shown to occur with time in individuals given regular doses of diuretics (86). The data of Edmonds & Wilson (87) strongly support the interpretation that such enhancement of potassium excretion results from increased secretion of aldosterone, and Edmonds (88) and Liddle (89) have shown that antagonists of aldosterone increase the excretion of sodium and diminish that of potassium when benzothiodiazine diuretics (hydroflumethiazide) are administered. In Edmonds' study (88) this was particularly marked in those patients previously showing the smallest excretion of sodium and the largest excretion of potassium in response to the diuretics.

In view of these considerations and the fact that no evidence has been adduced to indicate that benzothiazide diuretics have more than two specific actions—that on carbonic anhydrase and that responsible for their chloruretic effects, one may anticipate that once the chloruretic potency is great enough to yield maximum chloruresis without carbonic anhydrase inhibition, all of the drugs will have similar diuretic properties. One may, therefore, view with skepticism claims that among such potent chloruretic agents there are significant differences in their effects on potassium excretion (90, 91, 92).

Inferences concerning the locus of action of these diuretics in the renal tubule have been based on the application of two techniques, and unfortunately, the results obtained with the two procedures are almost diametrically opposed. Kessler and his associates (93) and Vander *et al.* (85) from studies using the stop-flow technique in dogs have concluded that the major effects of chlorothiazide on the reabsorption of sodium and chloride are exerted in the proximal convoluted tubule. (There is an additional distal tubule effect on pH, ammonia, and potassium excretion characteristic of carbonic anhydrase inhibitors.) On the other hand, a number of investigators have studied the effect of one or another diuretic of this group on the excretion of sodium, chloride, and water in man (94, 95, 96) and in dogs (97) undergoing water diuresis. The data obtained, particularly in the dog, strongly support the view that the major effect is upon the reabsorption of sodium and chloride in the distal system where sodium reabsorption leads, in water diuresis, to the production of dilute urine. The latter workers have found that in maximum water diuresis, the administration of thiodiazine

diuretics produces an increase of sodium and chloride excretion with essentially no change in urine flow. This is the behavior to be expected if the volume and salt content of fluid delivered to the site of dilution remains unchanged, but there is inhibition of the process of salt removal leaving water behind, which normally yields a dilute urine. These observations seem clearly to point to a site of action in the distal portions of the nephron—that is, the ascending limb of Henle's loop or beyond. The discrepancy seems to the reviewers to indicate the weakness of the stop-flow technique as a method for localizing activities in the renal tubule, particularly with regard to the more proximal of two or more processes effecting the transtubular movements of a substance. In the case of chlorothiazide, it would appear that the changes in distal tubule function produced by the drug are not sufficient to prevent the virtually complete removal of sodium during the prolonged period of stasis in stop-flow, but are sufficient to impair the removal of sodium as fluid from higher in the nephron passes this segment when flow is re-established.

A finding apparently related, at least in part, to the capacity of thiodiazine diuretics to impair urinary dilution is the interesting observation of Crawford & Kennedy (98, 99) that their administration to patients with diabetes insipidus leads to a marked reduction in urine flow and an increase in urine concentration. The increased concentration which occurs when the drug is first administered is quite in accord with its effects in normal animals in water diuresis (97). The subsequent decrease in urine flow is not so easily explained, but must be attributable to a decrease in the fraction of the glomerular filtrate escaping reabsorption in the proximal tubule. It would appear from studies of Earley & Orloff (86) that once a sodium deficit has been produced, the reduced urine volume and increased urine concentration can be maintained without further administration of drug, so long as the sodium deficit is maintained by a rigidly restricted salt intake; the changes in excretion are promptly reversed when the losses of salt have been restored. These results are in accord with the inferences of Havard & Wood (100) who considered the reduced urine flow to result from urinary salt loss and of Cutler *et al.* (101) who found that there was no decrease in urine volume when chlorothiazide was administered to diabetes insipidus patients if sufficient salt was administered to prevent negative balance. The proposal of Kennedy & Crawford (102) that the diuretics of this group produce their effects by a specific antagonism of the mineralocorticoids was presented largely without supporting data and is not easily reconciled with the findings of Earley & Orloff nor with those of Edmonds cited earlier (88).

The drugs as a group are rapidly absorbed from the gastrointestinal tract and rapidly excreted in the urine by a process involving active tubular secretion (74, 103). The latter process has been shown by stop-flow study to be localized in the proximal tubule (93) and to be inhibited by probenecid (74, 93) and, hence, presumably is that responsible for the secretion of

p-aminohippurate, diodrast, etc. Although earlier studies indicated that preventing the secretion of chlorothiazide did not reduce the diuretic effect when the drug was administered in large doses (74, 93), Beyer reports (104) that the action of the minimal effective dose is prevented by pretreatment with probenecid. This suggests that the concentration in the tubule lumen may be a critical factor in its activity.

The biochemical mechanism by which this group of compounds produces its effects is entirely unknown.

Mercurial Diuretics

The organic mercurials constitute the most effective diuretic agents known. Although the majority of those used clinically are substituted mercuripropyl derivatives with the following basic structure, R-CH_2-CHOY-CH_2-Hg-X (105, 106, 107), this configuration is not essential for diuretic activity; both mercuric chloride and mercuric cysteine (108) are diuretic as are certain organomercurials of dissimilar structure (109). The effect of variations in both the R and X groups which, in contrast to the OY group, may influence diuretic potency, solubility, and toxicity has been summarized in several articles (106, 107, 111). It is generally agreed that the terminal-C-Hg^+X^- group is largely responsible for diuresis, but whether this requires dissociation only to C-Hg^+ with one free mercury valence being made available (107) or rupture of the C-Hg bond releasing both valences (108) is the subject of much discussion (see below).

Mercury exerts its effect directly upon the renal tubular cells (112). Inhibition of sodium and chloride reabsorption follows accumulation of the derivative within the renal cortex (113, 114, 115). Chlormerodrin is secreted in the proximal nephron at a site co-extensive with that for PAH (116); it is probable that the other organic mercurials are also secreted by the same mechanism (117). Those which have been studied are excreted in the urine in large part as either the cysteine or acetylcysteine derivative of the parent compound (118, 119). The delay in diuresis which generally attends the administration of mercurials is not dependent upon a lag in either the uptake of mercury by the kidney or in its urinary appearance; considerable cortical accumulation (107, 114) as well as a high rate of excretion of the compound (115) may precede diuresis. Both Weiner *et al.* (120) and Campbell (117) consider that, in the chicken, mercury is transported into urine by a two-step process: tubule cell uptake which may be inhibited by probenecid and brom cresol green, and subsequent transfer into the urine.

Although probenecid interferes with both the excretion of mercury and its diuretic effect in the chicken (117), this has not been noted in other species. Kessler and associates (107) were unable to inhibit the secretion of *p*-chloromercuribenzoate, an inactive mercurial, with probenecid in the dog, and Weiner found no inhibition of the secretion of an active mercurial diuretic (119). A further demonstration of species differences is the obser-

vation that mercurials interfere with PAH transport in man, but not in the dog (121).

Inhibition of sodium and chloride reabsorption by mercurials is greater than that resulting from all other clinically useful diuretics (77). The increment in chloride excretion frequently exceeds that of sodium and is particularly notable in those edematous subjects in whom potassium loss in the urine is a prominent feature of the diuresis. This observation, as well as the occasional development of hypochloremic alkalosis following repeated injections of mercury, has led to the suggestion that the primary effect of the diuretic is on chloride transport, the diminution in sodium reabsorption being secondary to this phenomenon. Berliner (122) has pointed out the error in logic of this conclusion. The excess chloruresis observed is compatible with the view that proximal sodium reabsorption is primarily inhibited by mercurials, if one further assumes that the distal process whereby sodium is reabsorbed in exchange for potassium and hydrogen ions continues. Thus, if equivalent amounts of sodium and chloride are diverted from the proximal segment to the distal exchange site, one should expect chloride excretion to exceed that of sodium, the extra cation being made up by substitution of potassium, hydrogen, and ammonium ions. This would be particularly prominent in edematous subjects in whom the terminal exchange process is presumably stimulated by aldosterone. Predominant increases in potassium rather than sodium excretion are also observed with the chloruresis following administration of the thiazide derivatives in some edematous individuals and may be similarly interpreted (88). The observation of Giebisch (16) that chlormerodrin reduces the membrane potential of renal tubule cells in *Necturus* is consistent with, though not conclusive evidence for, the view that mercurials depress active sodium rather than chloride transport. Bisno *et al.* (123) have suggested that mercurials augment sodium excretion by increasing the passive flux of sodium from peritubular vessels into urine. Since the conclusion depends on interpretation of proximal tubule functions in stop-flow studies, it can not be accepted without reservation.

Mercurials inhibit the secretion of potassium in all species examined (31, 84, 124). In dog and man, if potassium excretion is initially elevated, mercurials uniformly lower the rate of excretion. On the other hand, if the initial rate of excretion is low, mercurial diuresis is associated with an increase in potassium excretion. The significance of this has been discussed in detail elsewhere (36, 84). Despite partial inhibition of the sodium-potassium exchange mechanism by mercury, the delivery of more sodium from the proximal segment to the exchange site provides the basis for increased secretion of potassium, albeit at less than the maximal rate.

Although it is generally agreed that changes in urine flow caused by mercurials are secondary to interference with solute reabsorption in the nephron, excluding a direct effect of the drug on water movement, consider-

able controversy exists as to the site of the diuretic effect. Depending upon the preconceptions of the investigator, the changes in urine flow induced by the mercurial during osmotic diuresis with and without antidiuretic hormone have been interpreted by some as deriving from the proximal nephron, by others from the distal nephron (94, 95, 125 to 133). Negative free water clearance has been reported to be unaltered by mercury in some studies, changed slightly in others. Similar results have been obtained with respect to the excretion of solute-free water.

Neither Miller & Riggs (131) nor Goldstein *et al.* (133) in carefully conducted studies observed any appreciable alteration in free water clearance during the superimposition of mercurials on maximal water diuresis in dog and man. The absence of a marked effect of mercurials on free water clearance contrasts with the results of studies with acetazoleamide and chlorothiazide. The administration of acetazoleamide is uniformly associated with an increase in free water clearance (134, 135), that of chlorothiazide with a fall (86, 94), indicating that they act on sodium transport at different sites in the nephron. The rise in free water clearance with acetazoleamide is indistinguishable from that observed during any nonspecific solute diuresis (136) indicating that there is no appreciable reduction of net solute reabsorption in those segments responsible for dilution of the urine. The increase in free water excretion is largely a consequence of a reduction in the back-diffusion of water attributable to the osmotic restraint of residual solute in the distal nephron (136, 137). The basis for the conclusion that chlorothiazide inhibits sodium chloride reabsorption in the diluting segment is discussed above. The mercurial effects are more difficult to interpret. Though consistent with a reduction in both proximal and distal reabsorption, the latter just sufficient to erase the increment in free water clearance generally provided by isosmotic solute diuresis, such a precise balance of the two effects is difficult to accept (133).

Diuresis caused by organic mercurials is strikingly potentiated by acidifying salts (138) and inhibited markedly by alkalinizing agents (139). Ethridge and his co-workers (139) demonstrated that the acidifying salts, ammonium chloride, ammonium nitrate, calcium chloride, and calcium nitrate, as well as phosphoric acid, when given in conjunction with salyrgan, effected a marked increase in the diuresis as compared with that due to the mercurial alone. In contrast the alkalinizing salts, potassium acetate, potassium bicarbonate, and sodium bicarbonate, all interfered with the diuretic response. The response was unmodified by the neutral salts, potassium chloride and sodium chloride.

Prior administration of sodium bicarbonate, though interfering with the natriuretic effect of mercurials, does not prevent the inhibition of potassium secretion (140). The potassium secretory system appears to be considerably more sensitive to mercury than is the system responsible for sodium chloride reabsorption since in normal dogs doses of mercury insufficient to increase sodium excretion diminish potassium secretion (141).

The increase in plasma chloride concentration, rather than changes in pH, has been thought to be responsible for the potentiating effect of acidifying agents (142). In support of this argument, Axelrod & Pitts (142) observed that respiratory acidosis, in contrast to metabolic acidosis, did not result in an augmentation of mercurial diuresis. Furthermore, the converse, hypochloremia, in edematous patients, is often associated with unresponsiveness to organic mercury. Although the filtered load of chloride may under certain circumstances condition the renal response to mercury, the "chloride load" hypothesis is inadequate to explain a number of observations. These have been discussed in detail by Levy *et al.* and Mudge & Weiner (108, 109). Potentiation occurs during chronic administration of ammonium chloride in the absence of significant hyperchloremia (143); ammonium nitrate augments the diuretic response despite absence of hyperchloremia (139); unresponsiveness to mercury is not observed when hypochloremia develops in hypokalemic alkalosis (144) but does occur in both metabolic (139) and respiratory alkalosis (145). Acetazoleamide which, in acute studies, does not appreciably affect the plasma chloride concentration has been reported to reduce the effectiveness of mercurials in both man (146) and dog (147). Finally, Levy *et al.* (108) have shown that sodium bicarbonate prevents mercurial-induced diuresis in dogs, even when the filtered load of chloride is artificially maintained at normal levels.

Most of the arguments relating to the site of action of mercurial diuretics have been based on histochemical data, alterations in water and electrolyte excretion, and effects on specific transport processes other than those for sodium and chloride in intact animals. Much of the earlier work has been reviewed in detail (109, 149) and will be commented on only briefly. It should be noted that neither the localization of a drug by histochemical techniques within a specific site in the nephron nor its interference with systems known to be present in specific segments, provides evidence regarding the site of inhibition of electrolyte transport by the agent in question. A drug may accumulate in a cell, enter the urine, and affect a luminal membrane transport process anywhere within the nephron. It may, after appearance in the urine, be reabsorbed more distally and exert its effect at another site. The available data relative to succinic dehydrogenase inhibition (150, 151, 152), sulfhydryl group concentration (153), and the transport of substances other than sodium and chloride (121, 125, 154) are probably of significance only with respect to the particular systems examined and may have no bearing on the diuretic action of the drug. Furthermore, conclusions relative to the site of action of mercurials based on the fraction of glomerular filtrate excreted in the urine are unwarranted, since partial inhibition of sodium transport throughout the nephron conceivably could result in similar data. On the other hand, there is no question that mercury exerts at least a portion of its effect in the distal segment in view of its interference with potassium secretion.

Vander *et al.* (148) and Kessler *et al.* (116) have utilized the stop-flow

technique in an effort to localize the site of action of mercury on sodium and chloride transport. Both groups have concluded that the major changes in water and electrolyte transport are limited to the proximal segment. This is based on two observations: (*a*) the minimal distal sodium concentration following mercury does not differ from that observed during control observations with mannitol alone, indicating to the investigators that distal sodium reabsorption is unaffected by mercury, and (*b*) the sodium concentration in the so-called proximal samples is greater with mercury than without, and the creatinine urine/plasma ratio is diminished, evidence of interference with both water and electrolyte reabsorption. The conclusion that the effect is exerted in the proximal tubule is based on the failure to demonstrate impaired sodium reabsorption in the distal tubule. However, the technique also failed to demonstrate that chlorothiazide inhibits distal sodium reabsorption, an action that fairly clearly exists (see above). As Kruhøffer (155) has stated, the findings in the distal effluent indicate that the concentration gradient against which sodium can be reabsorbed in the distal segment is not reduced by diuretic doses of mercury under the circumstances of the study, but do not prove that the reabsorptive capacity expressed as a rate is uninfluenced. Nevertheless, the contrast between the effects of mercurials and of chlorothiazide on water diuresis indicates considerable difference in their locus of action, and it appears fairly certain that a major part of the mercurial inhibition of sodium chloride reabsorption occurs in the proximal tubule. It may well be that partial inhibition of sodium transport throughout most of the nephron is effected.

The known affinity of inorganic mercury for sulfhydryl groups makes it probable that mercaptide formation is the principal reaction involving dissociable organic mercurials within the body. All active mercurials possess a C-Hg-X group which can provide at least one free Hg valence for this process; compounds of the structure R-C-Hg-C-R are not diuretic (149). The reversal and prevention of mercurial diuresis by the dithiol, 2,3-dimercaptopropanol (BAL), (156) and the failure of monothiols to do likewise (157) has been interpreted as indicating that the postulated sulfhydryl containing enzymes have a greater affinity for mercury than do the monothiols and a lesser affinity than do the dithiols (109, 149). The presumption is that BAL complexes with the mercurial *in vivo,* rupturing the inactive mercuri-mercaptide responsible for the diuresis.

Attractive as the sulfhydryl inactivation thesis may be, there is no direct evidence in support of it. There is little evidence that sulfhydryl-containing enzymes play a role in electrolyte transport. The early work implicating succinic dehydrogenase as the responsible energy source has been largely discarded (109, 158). Histochemical data correlating localized depression in sulfhydryl concentration within the nephron with mercurial administration are pertinent only to the extent (*a*) that blocking of sulfhydryl groups is responsible for the diuresis and (*b*) that blocking of sulfhydryl groups is limited to those involved in the transport process. On the other hand, the

lack of diuretic properties in *p*-chloromercuribenzoate (107), a known sulfhydryl inhibitor *in vitro,* does not exclude the participation of mercaptide formation, although it does exclude it as the sole factor responsible for diuretic activity. The recent observation (159) that *p*-chloromercuribenzoate both prevents and arrests diuresis resulting from active mercurials is strong evidence for sulfhydryl participation in the effect. If association or interaction with a receptor site adjacent to the sulfhydryl group is also necessary for diuresis, as suggested by both Mudge and Kessler and their associates (107, 109), formation of a *p*-chloromercuribenzoate mercaptide may prevent the active mercurial from engaging in this essential process.

Kessler *et al.* (107) and Mudge & Weiner (109) have proposed two alternative hypotheses concerning the mode of action of mercurials and the requirements for diuretic activity. Both groups consider accumulation, secretion, and mercaptide formation essential, although Mudge & Weiner caution against acceptance of the last as proven. Kessler *et al.* were able to show that the degree of accumulation of organic mercurials within the kidney varied and did not correlate with diuretic potency. They suggested that a specific steric configuration which permits a critical "lock and key" relationship between an active site in the kidney and the organic mercurial is a necessary requirement for diuretic potency. On the basis of an analysis of a number of organic mercurials, they concluded that diuretic activity requires that the compound contain a chain of not less than three carbons, a mercury atom at the end of the chain, and a hydrophilic group not less than three carbons away from the mercury. In their view, interference with electrolyte transport is dependent upon two conditions, combination of the free mercury valence of the terminal C-Hg^+ group with a sulfhydryl group and interaction of the hydrophilic group with an adjacent receptor site in the kidney. The thesis, which was recognized by the authors as being somewhat tenuous to the extent that they suggested an alternative hypothesis, is no longer acceptable. Numerous exceptions to the proposed configuration exist among organic mercurials, and mercuric chloride and mercuric cysteine are potent diuretics in the dog (109). Furthermore, the theory does not provide an explanation for the known effects of alterations in acid-base balance on the diuretic response.

Levy, Weiner & Mudge (108), on the other hand, concluded that diuretic potency requires rupture of the carbon-mercury bond, release of divalent mercury, and ionic interaction with two adjacent receptor sites within the kidney. They have suggested that the latter may involve association with a sulfhydryl group and an adjacent carboxyl or amino group. Since rupture of the carbon-mercury bond is a pH-dependent reaction, accelerated in acid solution, the release of ionic mercury within the body would be expected to be pH dependent. If the action of mercurials results from release of mercuric ion, rupture of the carbon-mercury bond would be accelerated in an environment of low pH either within the pertinent cells or the urine. In support of their theory they have observed that the diuretic

potency of both mercuric chloride and mercuric cysteine is unaffected by metabolic acidosis and alkalosis. All of the active organic mercurials which they studied liberate mercuric ions in an acid medium, whereas inactive mercurials with one exception do not. The acid-labile inactive mercurial did not appear in the urine following administration, suggesting that it was not transported to the proper place within the kidney to effect diuresis. Although they have been unable to detect inorganic mercury in the urine following injection of most of the active organic compounds, methodological limitations may account for this discrepancy, particularly since only a small fraction of the total mercury which accumulates in the kidney may be associated with the diuretic effect (115). Moreover, although most of the mercurials are excreted largely as the cysteine derivative of the parent compound (118), some, as is mercuric chloride, are excreted as mercuric cysteine (119). If this last is confirmed, it would constitute clear evidence of *in vivo* rupture of the carbon-mercury bond.

The hypothesis is attractive in that it suggests a reasonable explanation for the effects of alterations in acid-base balance on the diuretic response to the organic mercurials. It would be assumed that the enhanced diuretic activity in acidosis is attributable to acceleration of the production of ionic mercury by rupture of the carbon-mercury bond in an environment of lowered pH, whereas alkalosis would inhibit this reaction. However, it is difficult to specify an environment which undergoes pH change consistent in each case with the direction and magnitude of the modification of the diuretic effect. The plasma may be excluded since Mudge & Hardin (144) have shown that mercurial diuresis is not impaired in the alkalosis of potassium depletion, whereas the response to mercurials is depressed, markedly in man (146) and to a lesser extent in the dog (164), by acetazoleamide which has no effect on plasma pH (except for the reduction which results from impaired urinary acidification). Furthermore, it is not enhanced by respiratory acidosis which does reduce plasma pH (142, 160). Finally, Hilton (143) found that the potentiation produced by ammonium chloride was just as great when, as a result of compensatory mechanisms, plasma pH and bicarbonate had returned to normal as it was when the latter variables were low following the initial administration of ammonium chloride.

The possibility that it is modification of the pH of renal tubule cells which is critical to the efficacy of the mercurial diuretics is also not without objections, aside from the difficulty in specifying the extent and direction of changes in the intracellular pH. The pH of renal tubule cells is almost certainly lowered in respiratory acidosis which does not potentiate the action of mercurials (142, 160). The pH is also, in all probability, lowered in the alkalosis of potassium depletion (161, 162) which does not affect mercurial diuresis (144). On the other hand, the pH of tubule cells is probably more increased by the administration of potassium salts (163), which has not been found to suppress the effect of mercurials, than by the administra-

tion of sodium bicarbonate which has a very marked inhibitory action.

The possibility that the urine is the environment in which rupture of the carbon-mercury bond occurs is in accord with many but not all of the observations. The urine pH is not generally strikingly modified by either hypokalemic alkalosis or respiratory acidosis, nor is the diuretic response to mercurials. The urine pH is certainly elevated in alkalosis resulting from bicarbonate administration, and mercurial effects are suppressed. However, acetazoleamide is just as effective as the administration of bicarbonate in raising urine pH but much less so in preventing the action of organic mercurials.

This subject might be clarified by a careful quantitative re-evaluation of the effect of various conditions on the response to mercurial diuretics.

Cardiotonic Steroids

The cardiac glycosides are potent diuretic agents in congestive heart failure. Although both the glycosides and a number of the aglycones specifically interfere with cation transport in isolated tissues (166 to 169) and can be shown to exert direct renal tubular effects when injected into the renal circulation of experimental animals, they are not diuretic in the true sense in that their clinical effectiveness is limited to congestive heart failure. It is unlikely that the renal tubular effect demonstrated by Farber *et al.* (170) is a significant component of the diuretic action in heart failure. Such a thesis would presuppose unique changes in renal transport processes in congestive heart failure as contrasted with those in other conditions associated with edema (and in normals) which permit specific responsiveness to the digitalis-like derivatives. It is probable that diuresis, when observed, is secondary to changes in renal hemodynamic and cardiovascular function (171 to 173).

Despite this, the effects of these derivatives on electrolyte transport are of considerable interest. The drugs are important tools for examining the characteristics of cation transport. In a large variety of tissues it has been demonstrated that both the glycosides and, when studied, their aglycones specifically interfere with the active transport of sodium or potassium, or both. This was first established in the red cell by Schatzmann and has since been confirmed (165, 174 to 176) and extended to other tissues including the kidney (169). The molecular configuration necessary for activity has been studied in detail by Kahn & Acheson (175). The uniform interference with cation transport observed in the various tissues has led to the conclusion that all of the cells in question may transport cations by similar mechanisms, presumably involving linked exchange of sodium and potassium (177). Inhibition of potassium influx into red cells by the cardiotonic steroids is markedly diminished by increasing the concentration of potassium in the bathing medium (176). The kinetics of this process have been analyzed by Glynn (176) who considers the inhibition to be on a competitive basis. Inhibition of anion transport has also been observed; the accumula-

tion of PAH by renal cortical slices is diminished by strophanthidin (169), as is thyroidal iodide uptake (178). The inhibition in both instances appears to be related to the associated depression of cation transport since it can be minimized or prevented by increasing the potassium concentration of the medium and, in the case of renal cortical slices, reproduced by reducing intracellular potassium concentration by other means (169). The inhibition of chloride transport in gastric mucosa, on the other hand, may represent specific effects of the drugs on the chloride-transport process (179).

A direct renal tubular effect of digoxin was conclusively demonstrated by Hyman *et al.* (180). They observed a unilateral increase in sodium and water excretion following injection into the renal artery of the dog. These results have since been confirmed (181, 182). The observation by Schatzmann *et al.* (183) that ouabain interferes with net water movement in the proximal segment of *Necturus* is also interpreted as indicative of inhibition of sodium transport. Recently a detailed analysis of the effects of the aglycone, strophanthidin, on cation transport in the chicken kidney has been reported by Orloff & Burg (48). Injection of this compound into the renal portal venous circulation of one kidney results in a predominantly unilateral increase in sodium and chloride excretion and a fall in the excretion of potassium and hydrogen ions. Strophanthidin apparently interferes with all moieties of sodium reabsorption: reabsorption with chloride and exchange for potassium and hydrogen ions. The authors suggest that the effects are attributable to inhibition of the primary transport process which, in analogy to that proposed for frog skin (47), effects an exchange of sodium for potassium at the basal surface of the renal tubule cells.

In consonance with the observation of Glynn that the inhibitory effect of glycosides on red blood cells is reversed by increasing the potassium concentration of the medium, the diuretic effect of strophanthidin in the chicken and of ouabain in the dog is depressed by the administration of potassium salts (48, 181).

Wilbrandt has proposed (184) that the effect of digitalis-like substances is exerted through competition with adrenal mineralocorticoids, the latter acting as sites for the chelation of alkali metal ions on the membrane. Although this is an attractive hypothesis, Glynn (176) was unable to reverse the inhibition produced in human red cells by digoxin by the addition of large excesses of aldosterone and desoxycorticosterone, contrary to earlier findings (185). The effects of strophanthidin on the chicken kidney were not modified by large amounts of mineralocorticoids (48), although antagonistic effects in the rat have been reported (186).

The first metabolic effect of digitalis-like substances to be demonstrated was that strophanthidin had an ATP-sparing action in glucose-starved red blood cells (187). This is now known to be attributable to inhibition of an ATPase similar to that first found by Skou to be present in crab nerve (188) and since characterized in red blood cells by Post *et al.* (189). This ATPase requires sodium and potassium for activity, and its activity is

highly correlated with electrolyte transport in red cell ghosts (190). It seems probable that the renal effects are produced by a similar mechanism, and the presence in kidney of an enzyme with similar properties has been reported (191).

Antagonists of Aldosterone

Since the isolation and identification of aldosterone as the major mineralocorticoid of the adrenal (192), a tremendous literature has accumulated dealing with its physiologic effects, its role in various clinical disorders, and the factors governing its secretion. Several books and reviews may be cited (193 to 196). Space will not permit a review of the work relating to the effects of adrenal steroids on salt and water transport in the kidney. It is, however, pertinent to consider briefly the actions of a group of steroids which, because of their ability to reverse the actions of mineralocorticoids on renal sodium transport, have been introduced as possible diuretics.

The activities of the spirolactones, a series of compounds structurally related to aldosterone, were first described in 1957 by Kagawa *et al.* (197). These compounds have been shown to have the property of blocking, in competitive fashion, the effects of desoxycorticosterone and other mineralocorticoids on the excretion of sodium and potassium (198). Although there had been earlier instances reported in which steroids had been found to reverse the action of mineralocorticoids, in most of these studies it is highly probable that the effect of the antagonist was on glomerular filtration rather than transport by the tubules. This is clearly not the case with the spirolactones which, in general, have been found to have no effect on sodium or potassium excretion in the adrenalectomized or normal animal, but to decrease potassium excretion and increase sodium excretion in the presence of exogenous or endogenous mineralocorticoid (89, 197 to 199). Moreover, the effect of one of the series has been demonstrated in stop-flow studies in which glomerular filtration is excluded as a factor (200) and as an inhibitor of the effect of aldosterone on the sodium transport of isolated toad bladder (201). Thus, Vander *et al.* (200) were able, with SC 8109 [3-(3-oxo-17β-hydroxy-19-nor-4 androsten-17 α-yl) propionic acid γ-lactone], to reproduce in "normal" animals the modified stop-flow pattern which they found in adrenal insufficiency. It is noteworthy that this is the only instance in which such effects have been noted in animals neither given mineralocorticoids nor stimulated to secrete aldosterone by a low salt diet. However, the rather traumatic procedure involved in the surgical preparation for the stop-flow is sufficient to induce appreciable aldosterone secretion (202), and it is possible that some contraction of extracellular fluid may have been induced by the massive mannitol diuresis used for stop-flow studies.

In clinical use as diuretics, the spirolactones have proven considerably less effective than a number of other diuretic agents and their chief value seems to be in those relatively resistant patients in whom the administration of benzothiazide or mercurial diuretics produces a marked increase in

potassium excretion (89). In such individuals it has been assumed that the electrolyte excretion pattern is a manifestation of markedly increased aldosterone effects on sodium-potassium exchange, and this hypothesis appears to be confirmed by the fact that the excretion of potassium may be suppressed and that of sodium enhanced by the simultaneous administration of spirolactones.

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