MICROPUNCTURE AND DIURETICS[1,2,3]

By John H. Dirks and John F. Seely

McGill University Clinic, Department of Medicine, Royal Victoria Hospital, Montreal, Canada

The action of diuretics has been extensively investigated in recent years by means of micropuncture methods. The results of such studies have helped considerably to shape present understanding of salt and water reabsorption by the renal tubule. This review will attempt to deal solely with all of the pertinent micropuncture data, since more comprehensive reviews of other aspects of diuretics are readily available (1, 2). Micropuncture studies have naturally focused on two major aspects of diuretic agents, namely, the site and mechanism of their action. A third important aspect of such studies has been the nature and extent of various intrarenal mechanisms which modify primary changes in tubular reabsorptive capacity. Before outlining the results that have been obtained for each of the various diuretic agents, we propose firstly to consider some of the methods used in such studies and their limitations, and secondly to review some recent advances in our concepts of sodium reabsorption and its control mechanisms where they are relevant to the interpretation of diuretic studies.

Micropuncture methodology.—The most important measurement in the overall assessment of sites of diuretic action is the determination of the ratio of the inulin concentration in the tubule fluid to that of the plasma (TF/P), which is used to measure fractional fluid reabsorption (1-P/TF). This determination, however, carries a number of inherent limitations, for the combined analytical and sampling error alone may lead to errors in the estimation of fractional reabsorption in the proximal tubule as large as ± 10 per cent (3). This, in many instances, exceeds the overall magnitude of fractional sodium excretion. Artifacts associated with the technique of collection are a serious potential hazard. The dangers of altered flow proximal to the puncture site as a result of tubular obstruction or excessive suction, as well as of retrograde fluid collection from distal points, must be avoided. Another limitation has been the difficulty of obtaining valid control data for comparison with a set of experimental data. The scatter of results when TF/P ratios are plotted against tubular distance, plus the variations in the state of body hydration which can influence TF/P ratios, make it hazard-

[1] The survey of literature pertaining to this review was concluded in July 1968.

[2] Abbreviation used in this review: TF/P (concentration in the tubule fluid/ concentration in plasma).

[3] This study was supported by the Medical Research Council of Canada.

ous to draw valid conclusions from the results of separate experiments. The recollection technique (4) has been a significant improvement, since each tubule serves as its own control. The recollection of proximal tubule fluid samples from the same puncture site, as long as several hours apart, of itself does not significantly alter fractional reabsorption. Since small changes in fractional reabsorption at a proximal site result in large changes in the volume presented to more distal sites, changes in distal TF/P inulin ratios must be interpreted in the light of proximal events.

While free-flow inulin concentration ratios are important for the determination of net effects at various sites within the nephron, they give no detailed information about transport processes at a tubular level. A recent method, which has been extensively applied for the measurement of intrinsic reabsorptive capacity of the tubule, has been the split-oil-droplet technique (5). Gertz has shown that the reabsorptive capacity of the proximal tubule may be expressed as a rate constant per unit volume ($C/\pi r^2$), which applies equally to fractional fluid reabsorption during stationary (split-oil-droplet) or free-flow conditions as a function of contact time (6). The free-flow contact time is usually measured by the transit time for a bolus of lissamine green dye to travel from glomerulus to puncture site (7). A number of observers have pointed out that the diameter of the tested tubule appears to be markedly dilated during split-droplet studies (8-10), but the significance of this is not clear. Furthermore, lissamine green dye is not entirely innocuous since its repeated use may lead to transient collapse of the kidney. In spite of these reservations, the validity of the half-time measurement has generally been supported by the accuracy with which fractional reabsorption in the proximal tubule under free-flow conditions may be predicted from the results of split-droplet half-time studies and transit time (6, 9, 11, 12). Hence, fractional reabsorption, at least in the proximal tubule, may be regarded as the outcome of two sets of factors; those controlling the reabsorptive capacity of the tubule, and those determining the contact time. In assessing the action of a particular diuretic agent, it is important to distinguish between effects on fractional reabsorption and effects on reabsorptive capacity of the tubule per se, since the two need not vary in a predictable fashion.

Since free-flow TF/P sodium ratios as well as those of potassium, calcium, magnesium, and osmolality are normally close to unity throughout the proximal tubule, net changes in electrolyte reabsorption in this segment can be equated with net changes in fluid reabsorption measured by means of TF/P inulin ratios. Changes at a proximal site in these electrolyte concentration ratios with diuretic agents, apart from osmotic diuretics, are unlikely, but have been incompletely explored. Steady state TF/P ratios for sodium and potassium significantly below one are observed in the proximal tubule only in the presence of sufficient amounts of nonreabsorbable solute, such as mannitol or raffinose, to maintain TF/P osmolality ratios at unity. Under these conditions at zero net flux, TF/Pratios for both these elec-

trolytes approximate 0.7 (12–16) and thus are a measure of the maximum gradient against which an active ion transport pump can work. This method of stopped-flow microperfusion has been employed in a number of studies on the mechanism of diuretic action.

Free-flow TF/P electrolyte ratios in the early distal convoluted tubule are normally less than one, reflecting the normal hypotonicity at this site. Changes in the free-flow concentration ratios for sodium are readily observed with many diuretics and have been used to infer effects of these agents on sodium transport within the ascending limb of Henle's loop. The concentration of sodium in the tubular fluid emerging from the loop of Henle, however, is liable to be influenced by a number of other variables, including flow rate (17, 18), the medullary gradient (19), and the concentration of nonreabsorbable solute such as urea in tubular fluid (11, 19). Hence, changes in the free-flow TF/P sodium ratios by themselves do not provide conclusive evidence for an inhibitory effect of a diuretic agent on tubular transport within the loop of Henle and distal tubule. Split-droplet studies in the distal convoluted tubule cannot be interpreted precisely, in the absence of the sodium concentration within the reabsorbed droplet. A better assessment is provided by measures of steady state concentration ratios at zero net flux. Net effects on electrolyte transport at distal sites in the nephron can be inferred from the TF/P sodium or potassium ratio divided by its corresponding inulin ratio. If any additional excretory effects have occurred, it is presumed that further inhibition has taken place in the collecting ducts. An implicit assumption of this analysis is that sampling of surface nephrons is a valid representation of both surface and deeper cortical nephrons. This assumption is in urgent need of some direct experimental testing in the *in vivo* kidney. The problem of puncturing deep cortical nephrons in a physiological state, however, is at present insurmountable.

Physiological considerations relevant to diuretic action.—The forces that govern sodium and water reabsorption have been studied in some detail within the proximal tubule for a variety of reasons. Since proximal events must always be known to allow proper interpretation of more distal changes, it is understandable that this segment should receive the greatest attention. Diuretics exert their effects independently of changes in filtration rate and are generally assumed to interfere in some way with active sodium transport at a tubular level. Studies under a variety of experimental conditions have shown that reabsorptive rate in the proximal tubule is adjusted proportionately to changes in the filtered load, tending to maintain constancy of fractional reabsorption (4, 9, 18, 20–26). The degree of constancy and the underlying mechanisms responsible for this phenomenon of glomerulotubular balance are currently in dispute. It is presumed that a similar balance holds during diuretic conditions, although there are no studies to prove the point.

The most intensively studied of the various factors known to affect transport capacity of the proximal tubule has been the state of extracellular

fluid volume expansion. Micropuncture studies during volume expansion have shown that fractional reabsorption is markedly decreased in the proximal tubule (4, 8, 11, 27–29) chiefly as a result of an inhibition of intrinsic tubular reabsorptive capacity (8, 11, 29, 30), which may (8, 29, 30) or may not (11) be augmented by decreases in transit time. Recent evidence points to the existence of a new hormone which mediates this response (31–34). Rapid depletion of extracellular fluid appears to have the opposite effect of augmenting fractional reabsorption in the proximal tubule (26). Fluid volume is therefore a critical variable which must be carefully controlled if incidental effects owing to body hydration are not to be confused with primary effects of diuretic agents.

A number of investigators have recently focused on the role of hemodynamic factors in determining the reabsorptive rate of the proximal tubular epithelium. Lewy & Windhager (9) observed that the reabsorptive half-time was significantly reduced in the rat proximal tubule during partial renal venous occlusion and that the degree of reduction was significantly correlated with decreases in renal plasma flow. They have suggested that the net rate of transepithelial transport is coupled in some way with the removal of tubular reabsorbate from the interstitial space into the peritubular capillary bed. Earley (35) has marshalled considerable data from indirect clearance experiments which are consistent with this hypothesis. Micropuncture observations on the decreased fractional reabsorption of the proximal tubule which occurs during selective renal vasodilatation (36, 37) and during increases in renal perfusion pressure (38) are also consistent with this view. It is apparent that many diuretic agents are associated with striking hemodynamic changes within the kidney (39–41). The extent to which these changes may contribute to the overall diuresis has never been critically examined. The separate hydrodynamic factors that together determine tubular size, flow velocity, and hence transit time in the proximal tubule are poorly understood, probably in part because of the present inability to measure accurately small differences in hydrostatic pressures within the renal tubules and capillary bed.

Precise knowledge of the control mechanisms for fluid reabsorption at other sites of the renal tubule is still largely fragmentary. Net sodium transport within the loop of Henle appears to be limited only by the transtubular concentration gradient which may be maintained, and by the relative impermeability to water which normally characterizes this site. The establishment of transtubular concentration gradients for sodium has been shown to be a critical function of flow rate. Reductions in flow rate produced by renal arterial clamping led to a disproportionate increase in fractional reabsorption of water compared with that of sodium, resulting in elevations of early distal TF/P sodium ratios (18). Similar changes in sodium ratios were also observed when single loops were perfused at higher than normal flow rates (17). Furthermore, particularly in the rat, maximal concentration gradients can only be observed in the presence of significant

amounts of nonreabsorbable solute normally provided by urea within the tubular lumen (11, 19).

Apart from the well-known effects of aldosterone on distal tubule sodium transport (15), there is little exact knowledge of other control mechanisms at this site. The role of hemodynamic and other factors that determine flow velocity and tubular dimensions has not been clarified. Landwehr, Klose & Giebisch showed that the equilibrium concentration ratio for sodium was unaltered by isotonic saline loading in the rat, suggesting that the strength of the sodium pump was uninhibited (11). The absolute rate of sodium reabsorption, however, appears to be limited and fails to increase in response to a greater load (11, 42). A similar imbalance between load and reabsorptive capacity in the distal tubule, under diuretic conditions, may be important for net effects when proximal or loop transport is inhibited. The concept of nephron heterogeneity as an important factor in determining the total reabsorptive capacity of the distal tubule has recently been raised by Rector et al. (32). They have proposed that volume expansion leads to a redistribution of filtrate from deep nephrons with "high capacity loops" to superficial nephrons with "low capacity loops." This aspect of renal function is in need of direct experimental proof before functional models derived from micropuncture experiments can be unreservedly applied to overall tubule function, specifically during diuretic action.

In the following sections, we propose to deal with each of the various diuretic agents in turn: mannitol, carbonic anhydrase inhibitors, mercurials, thiazides, and furosemide and ethacrynic acid. The latter two will be considered together, in view of their highly similar pharmacologic effects.

Mannitol.—Mannitol has been studied extensively in both free-flow micropuncture and stopped-flow microperfusion experiments and has been particularly important in establishing the active nature of sodium transport. The potent diuretic properties of mannitol infusions have generally been attributed to its osmotic properties acting principally in the proximal tubule (43). Early micropuncture studies in the *Necturus* kidney by Windhager et al. (44) established the isosmotic nature of proximal reabsorption during perfusions with varying concentrations of mannitol. They also showed that net salt and water transport decreased linearly with decreasing concentrations of sodium chloride in the perfusion solution to which sufficient mannitol had been added to bring total osmolality equal to that of plasma. In this way, they were able to determine a limiting concentration gradient for sodium at zero net flux. A limiting gradient for sodium and potassium of approximately equal magnitude was subsequently demonstrated in the proximal tubule of the rat (12–16). No such studies are available as yet in the dog or higher species, though it seems highly probable that a similar gradient will be found.

Free-flow micropuncture studies in the rat during hypertonic mannitol infusions have also demonstrated the isosmotic nature of proximal reabsorption (45, 46), as well as that TF/P sodium ratios fall significantly

below unity and approach the value of the limiting gradient. Windhager & Giebisch (47) found that the mean TF/P sodium ratio in the mid proximal tubule was 0.84, and from their inulin ratios they estimated a 20 per cent reduction in net water reabsorption in comparison with earlier published data (48). Ullrich et al. (46) confirmed the fall in free-flow TF/P sodium ratios, but their limited data allow no conclusions to be drawn with regard to net effects on fluid reabsorption. Free-flow micropuncture studies in the dog (26), using the recollection technique, showed that net proximal reabsorption was readily inhibited by massive mannitol infusions when urine volumes exceeded 10 per cent of the filtration rate. Smaller loads failed to show significant inhibitory effects at a fractional excretion rate of less than 4 per cent.

The cited studies in the rat (46, 47) had established that distal TF/P sodium ratios decreased further during mannitol diuresis and indicated that increased sodium reabsorption at this site may compensate in part for a reduction in proximal sodium reabsorption. Since distal TF/P sodium ratios in the dog are probably close to the limiting gradient during antidiuretic conditions (49), additional net effects in this species within the distal tubule and collecting duct, owing to mannitol infusions, are likely, but have not been conclusively demonstrated.

Gottschalk & Mylle (50) observed that proximal and distal intratubular hydrostatic pressures were elevated during mannitol diuresis and that the normal pressure gradient between the proximal and distal tubule was abolished. Moreover, since the rise in intratubular pressure correlated directly with increases in urine flow, they demonstrated that the collecting duct was the ultimate site of resistance to flow within the nephron. Koch et al. (51) have recently measured glomerular capillary pressure by an indirect method (52) under various diuretic conditions. They found that mannitol led to increases in intratubular free-flow pressure which accounted for a fall in glomerular filtration rate, since glomerular capillary pressure failed to increase.

In summary, there is little doubt that massive mannitol infusions lead to significant inhibition of fractional reabsorption in the proximal tubule. It is not clear, however, whether more modest loads, as are frequently used clinically, have major inhibitory effects within the proximal tubule. Significant effects at more distal sites along the nephron seem likely, but are in need of experimental validation. The role played in the overall diuresis by fluid volume changes and renal hemodynamic alterations produced by mannitol infusions, has never been critically examined.

Carbonic anhydrase inhibitors.—This group of agents is among the weakest of diuretics employed therapeutically, but has been studied extensively and in great detail by micropuncture techniques owing to the effects of these diuretics on renal acidification and tubular electrolyte reabsorption. The principal body of micropuncture studies on the effects of these diuretics deals with mechanisms of tubular acidification and bicarbonate reabsorption

and is covered at length in a recent review by Maren (53). Studies in both the rat and dog (54–57) have shown that the administration of acetazolamide leads to significant elevations in the TF/P bicarbonate ratios greater than unity in the proximal tubule during free-flow conditions, with reciprocal reductions in TF/P chloride ratios. This effect is generally attributed to inhibition of hydrogen ion secretion into the lumen of the proximal tubule, resulting from reduced cellular proton supply when the catalytic activity of carbonic anhydrase is blocked. It is currently thought that outward sodium transport is partially coupled in some way to hydrogen ion secretion. Inhibition of the latter, brought about by carbonic anhydrase inhibitors, could thus lead to a reduction in net sodium and water reabsorption in the proximal tubule.

Conclusive evidence, however, for reduced fractional fluid reabsorption is still lacking. Dirks, Cirksena & Berliner (26) found that TF/P inulin ratios tended to decrease after acetazolamide, though not significantly when compared to control collections. Free-flow studies in the rat by Meng (58) showed that no significant change in fractional sodium reabsorption occurred after acetazolamide. Weinstein (56) recently found that fractional fluid reabsorption increased by 25 per cent after acetazolamide, while filtration rate fell to a similar extent. Volume losses do not appear to have been replaced and may have accounted for his results. A significant increase in steady state proximal sodium ratios after acetazolamide has recently been cited (59).

In the distal convoluted tubule, free-flow studies in both dog and rat have revealed that acetazolamide led to elevated concentrations of bicarbonate (57, 60, 61). Potassium concentration ratios are also increased (62), which can be attributed to an increased negativity of the transtubular potential (16, 62–64). Meng (58) found that TF/P sodium ratios are significantly higher than controls and remained so throughout the distal tubule. Analysis of his inulin data revealed that the major inhibitory effects occurred within the ascending limb of the loop of Henle and could account quantitatively for the overall natriuresis that was observed. In contrast to these studies in the rat, Clapp & Robinson (65) failed to find any significant effects on TF/P osmolality ratios in the dog after acetazolamide. Whether this indicates a species difference, or is only the result of different experimental conditions, is not clear.

In summary, it is evident that carbonic anhydrase inhibition diminishes hydrogen ion secretion and bicarbonate reabsorption throughout the nephron. Conclusive evidence for reduced fractional sodium and water reabsorption within the proximal tubule has not been obtained, and the absence of net effects may indicate preferential reabsorption of sodium with chloride rather than bicarbonate. Significant inhibition of fractional reabsorption has been observed in the ascending limb of Henle's loop in the rat.

Mercurials.—Mercurial diuretics have been relatively neglected by those studying renal function with micro methods in preference to studies em-

ploying newer and more rapidly acting agents. Clearance studies, on the other hand, are numerous and frequently conflicting in their conclusions (2). Giebisch has reported (66) that chlormerodrin resulted in a lowering of the transcellular electrical potential across the peritubular membrane of the proximal tubule in the *Necturus* kidney. No further results of the effects of mercurials on tubular potentials have been published and the significance of this observation is not clear. Dirks, Cirksena & Berliner (26) were unable to show any inhibitory effects within the dog proximal tubule following the administration of diuretic doses of chlormerodrin. In fact, they observed a significant enhancement of proximal reabsorption which they attributed to volume depletion occurring as a result of unreplaced urinary losses. This interpretation was supported by the finding that when losses were met with quantitative saline replacement the degree of proximal enhancement was much less marked.

Clapp & Robinson (65) reported that chlormerodrin administration led to a rise in TF/P osmolality ratios in the distal tubule of the dog, suggesting an action within the ascending limb of the loop of Henle, since sodium with its attendant anions provides the major contribution to the total osmolality of the distal tubule fluid in this species (49). In the absence of inulin data, net effects cannot be established with certainty, nor can effects of flow rate through the limb of Henle be excluded. There are no reported studies on the effects of mercurials on steady state concentration ratios for sodium at zero net flux in either the proximal or distal tubule. There are also no direct studies on the changes in fractional reabsorption at distal sites in the nephron. It is evident that precise knowledge of the site and mechanism of this potent group of diuretics must await further studies. From the available data, it is quite likely that major inhibitory effects occur in the loop of Henle.

Thiazides.—The thiazide group of diuretics has been the object of numerous micropuncture experiments. Ullrich (67) has shown that the half-time for reabsorption of a saline droplet in the rat proximal tubule was significantly prolonged by chlorthalidone. He and his co-workers (68), using microperfusion techniques, subsequently reported more detailed studies on unidirectional sodium fluxes across the proximal and distal tubules of the rat nephron. They found that chlorthalidone led to significant reductions in active sodium efflux rates at both sites, while passive influx rates remained unaltered. These results were supported by their finding that steady state concentration ratios in the proximal and distal tubule were both increased, and appear to provide conclusive evidence for inhibitory effects of chlorthalidone on active sodium transport at proximal and distal sites of the nephron.

Free-flow micropuncture studies suggest that these tubular inhibitory effects are translated into net effects only at distal sites of the nephron. Free-flow studies in the dog (26) and rat (58) have shown no decreases in fractional reabsorption in the proximal tubule. In contrast, analysis of early distal tubule samples in the latter study revealed striking reductions both in

fractional water and sodium reabsorption in the loop of Henle, as evidenced by significantly higher TF/P sodium ratios and higher sodium/inulin TF/P ratios. The augmented load presented to the distal tubule exceeded the net increase in urinary excretion of sodium and water. This work has in part been confirmed by Thurau (69) who observed that TF/P sodium ratios in the distal tubule were elevated after hydrochlorothiazide, and by Clapp & Robinson (65) who showed that the TF/P osmolality ratios in the early distal tubule of the dog were significantly higher after chlorothiazide. Meng (58) also noted that distal TF/P potassium ratios were significantly elevated by thiazide administration, which accounts for the kaliuretic effects of these drugs. The mechanism for this is unclear and may indicate an inhibitory effect on potassium reabsorption. It is also possible that this may have resulted from an increase in the distal transtubular negative potential. Krause et al (70) have shown that hydrochlorothiazide administration to rats led to a fall in the effective filtration pressure and hence filtration rate, since glomerular capillary pressure was unaltered while intratubular pressure rose with increases in urine volume. The bulk of micropuncture evidence, in sum, supports the view gained from previous clearance experiments that these agents act predominantly within the diluting segment of the limb of Henle for their net effects.

Furosemide and ethacrynic acid.—Furosemide has been studied in great detail by the use of micropuncture techniques. Ethacrynic acid has been studied far less, perhaps owing to its ineffectiveness as a diuretic in the rat, although there appear to be few if any significant differences in the pharmacological effects of these two drugs in the dog. The results that have been obtained for both agents closely resemble those of the thiazide group in many respects. The stationary microperfusion studies of Ullrich (68) showed that furosemide led to significant inhibition of sodium efflux rates in both proximal and distal tubules of the rat nephron, and that steady state concentration ratios for sodium were significantly higher at both sites. Since unidirectional fluxes of sodium and chloride were also unaltered (71), he and his co-workers concluded that furosemide acted directly on the active sodium transport mechanism rather than on passive permeability characteristics of the tubular cell. Further evidence for inhibitory effects on sodium transport capacity was provided by the study of Rector et al. (3, 29) in which significant prolongation of the split-droplet half-time was observed in the proximal tubule of the rat.

Studies under free-flow conditions by several groups of investigators have shown no net inhibition within the proximal tubule. In the study of Dirks, Cirksena & Berliner (26), both furosemide and ethacrynic acid led to significant increases in fractional reabsorption, presumably owing to volume losses. When saline was given to replace urinary losses during administration of ethacrynic acid, no significant alteration in fractional reabsorption could be found, despite fractional excretion rates as high as 25 to 30 per cent for sodium and water. Bennett, Brenner & Berliner (72) have subsequently also reported that furosemide failed to alter significantly frac-

tional reabsorption in the monkey proximal tubule, despite saline replacement of volume losses. Deetjen (73) noted that proximal TF/P inulin ratios in rats were unchanged as long as the filtration rate remained in the control range. Animals whose filtration rate was reduced by 40 per cent or more as a result of unreplaced volume losses, showed decreases in fractional reabsorption. Rector et al. (3, 29) showed in their study that furosemide did not inhibit proximal fractional reabsorption in the rat as a result of an increase in proximal transit time, owing to tubular dilatation. They were also able to show that the reabsorptive rate constant calculated from inulin and transit-time data was clearly reduced and agreed with their direct estimate of the reabsorptive rate constant obtained from the split-droplet technique. In contrast, two additional studies in the rat (58, 74) have both suggested that furosemide results in a slight degree of inhibition of fractional reabsorption in the proximal tubule.

There is a considerable body of evidence which now points to the water-impermeable sites of the nephron as the principal site of action of these two drugs. Micropuncture studies in the rat, dog, and monkey have all shown that furosemide results in striking elevations of distal tubule TF/P sodium ratios under free-flow conditions (58, 49, 72) as well as TF/P osmolality ratios in the latter two species (65, 72). Malnic, Vieira & Enokibara (74) showed that the TF/P chloride ratios in the rat were raised to a similar extent by furosemide. They also made the important observation that distal transtubular potentials were not significantly changed. This is of interest since the studies of Bennett et al. in the dog and monkey (49, 72) have shown that the distal TF/P potassium ratios were significantly higher than those of control animals. If the potential difference is unaltered by furosemide in these species as well, the results of these studies suggest that furosemide inhibits active potassium transport at the luminal membrane.

In view of the effect of these agents on patients with diabetes insipidus, Ullrich has investigated the effect of furosemide on water permeability in the distal tubule of diabetes insipidus rats and this was not found to be significantly altered (71). Krause et al. (70) studied the effects of furosemide on hydrostatic pressures within the rat nephron and found, in contrast to their observations cited previously for mannitol and thiazides, that the glomerular capillary pressure increased along with the intratubular pressure. The filtration rate, as could be predicted, remained unchanged or increased slightly.

In summary, these results support the view that furosemide exerts predominant inhibitory effects within the ascending limb of the loop of Henle and distal tubule. It is apparent that sodium transport capacity is also inhibited in the proximal tubule but, owing to the ensuing tubular dilatation and prolongation of reabsorptive time, these effects appear to be effectively cancelled out. The close similarity of the effects of ethacrynic acid in clearance experiments in the dog suggests a similar action to that of furosemide in this species.

Conclusions.—Micropuncture studies have supplied much of the critical information concerning the site and mechanism of diuretic action. It is clear, moreover, that such studies must be continually reinterpreted in the light of changing physiological concepts of the renal tubule. Primary effects of diuretic agents must be distinguished from the complex train of compensatory changes which they induce. New technical advances will greatly aid in detecting the smaller net effects of the weaker diuretics. This review indicates much additional scope for new studies of diuretic action at all nephron sites, with a variety of techniques, which should extend and solidify the evidence described, as well as detail the transport effects of agents not yet studied.

LITERATURE CITED

1. Seldin, D. W., Ed., *Ann. N.Y. Acad. Sci.*, **139,** Art. 2, 273–539 (1966)
2. Bank, N., *Ann. Rev. Med.*, **19,** 103–18 (1968)
3. Rector, F. C., Jr., Brunner, F. P., Sellman, J. C., Seldin, D. W., *Ann. N.Y. Acad. Sci,* **139,** Art. 2, 400–07 (1966)
4. Dirks, J. H., Cirksena, W. J., Berliner, R. W., *J. Clin. Invest.*, **44,** 1160–70 (1965)
5. Gertz, K. H., *Pflüger's Arch. Ges. Physiol.*, **276,** 336–56 (1963)
6. Gertz, K. H., Mangos, J. A., Braun, G., Pagel, H. D., *Pflüger's Arch. Ges. Physiol.*, **285,** 360–72 (1965)
7. Steinhausen, M., *Pflüger's Arch. Ges. Physiol.*, **277,** 23–35 (1963)
8. Hayslett, J. P., Kashgarian, M., Epstein, F. H., *J. Clin. Invest.*, **46,** 1254–63 (1967)
9. Lewy, J. E., Windhager, E. E., *Am. J. Physiol.*, **214,** 943–54 (1968)
10. Gottschalk, C. W., *Harvey Lectures, Ser. 58,* 99–124 (1963)
11. Landwehr, D. M., Klose, R. M., Giebisch, G., *Am. J. Physiol.*, **212,** 1327–33 (1967)
12. Hayslett, J. P., Kashgarian, M., Epstein, F. H., *J. Clin. Invest.*, **47,** 774–82 (1968)
13. Kashgarian, M., Stöckle, H., Gottschalk, C. W., Ullrich, K. J., *Pflüger's Arch. Ges. Physiol.*, **277,** 89–106 (1963)
14. Giebisch, G., Klose, R. M., Malnic, G., Sullivan, W. J., Windhager, E. E., *J. Gen. Physiol.*, **47,** 1175–94 (1964)
15. Hierholzer, K., Wiederholt, M., Holzgreve, H., Giebisch, G., Klose, R. M., Windhager, E. E., *Pflüger's Arch. Ges. Physiol.*, **285,** 193–210 (1965)
16. Malnic, G., Klose, R. M., Giebisch, G., *Am. J. Physiol.*, **211,** 548–59 (1966)
17. Cortney, M. A., Nagel, W., Thurau, K., *Pflüger's Arch. Ges. Physiol.*, **287,** 286–95 (1966)
18. Landwehr, D., Schnermann, J., Klose, R. M. Giebisch, G., *Federation Proc.*, **26,** 547 (1967)
19. Giebisch, G., Windhager, E. E., *Am. J. Med.*, **36,** 643–69 (1964)
20. Glabman, S., Aynedjian, H. S., Bank, N., *J. Clin. Invest.*, **44,** 1410–16 (1965)
21. Rector, F. C., Jr., Brunner, F. P., Seldin, D. W., *J. Clin. Invest.*, **45,** 590–602 (1966)
22. Brunner, F. P., Rector, F. C., Jr., Seldin, D. W., *J. Clin. Invest.*, **45,** 603–11 (1966)
23. Steinhausen, M., *Bibliotheca Anat.*, **9,** 135–40 (1967)
24. Windhager, E. E., *Physiologist,* **11,** 103–14 (1968)
25. Brenner, B. M., Bennett, C. M., Berliner, R. W., *J. Clin. Invest.*, **47,** 1358–78 (1968)
26. Dirks, J. H., Cirksena, W. J., Berliner, R. W., *J. Clin. Invest.*, **45,** 1875–85 (1966)
27. Cortney. M., Mylle, M., Lassiter, W. E., Gottschalk, C. W., *Am. J. Physiol.*, **209,** 1199–1205 (1965)
28. Watson, J. F., *Am. J. Physiol.*, **210,** 781–85 (1966)
29. Rector, F. C., Jr., Sellman, J. C., Martinez-Maldonado, M., Seldin, D. W., *J. Clin. Invest.*, **46,** 47–56 (1967)
30. Heller, J., Novakova, A., *Experientia,* **23,** 799–801 (1967)
31. Davis, J. O., Johnston, C. I., Howards, S. S., Wright, F. S., *Federation Proc.*, **26,** 60–69 (1967)
32. Rector, F. C., Jr., Martinez-Maldonado, M., Kurtzman, N. A., Sellman, J.

C., Oerther, F., Seldin, D. W., *J. Clin. Invest.*, **47,** 761–73 (1968)
33. Lichardus, B., Pliska, V., Uhrin, V., Barth, T., *Lancet*, **I,** 127–29 (1968)
34. Cort, J. H., Pliska, V., Dousa, T., *Lancet*, **I,** 230–31 (1968)
35. Earley, L. E., *Ann. N.Y. Acad. Sci.*, **139,** Art. 2, 312–27 (1966)
36. Seely, J. F., Dirks, J. H., *Clin. Res.*, **15,** 478 (1967)
37. Hayslett, J. P., Kashgarian, M., Epstein, F. H., *Clin. Res.*, **16,** 385 (1968)
38. Koch, K. M., Aynedjian, H. S., Bank. N., *Clin. Res.*, **15,** 480 (1967)
39. Stahl, W. E., *New Engl. J. Med.*, **272,** 381–86 (1965)
40. Goldberg, A. H., Lilienfield, L. S., *Proc. Soc. Exptl. Biol. Med.*, **119,** 635–42 (1965)
41. Birtch, A. G., Zakheim, R. M., Jones, L. G., Barger, A. C., *Circulation Res.*, **21,** 869–78 (1967)
42. Lassiter, W. E., Mylle, M., Gottschalk, C. W., *Am. J. Physiol.*, **206,** 669–73 (1964)
43. Wesson, L. G., Anslow, W. P., Jr., *Am. J. Physiol.*, **153,** 465–74 (1948)
44. Windhager, E. E., Whittembury, G., Oken, D. E., Schatzmann, H. J., Solomon, A. K., *Am. J. Physiol.*, **197,** 313–18 (1959)
45. Gottschalk, C. W., Mylle, M., *Am. J. Physiol.*, **196,** 927–36 (1959)
46. Ullrich, K. J., Schmidt-Nielsen, B., O'Dell, R., Pehling, G., Gottschalk, C. W., Lassiter, W. E., Mylle, M., *Am. J. Physiol.*, **204,** 527–31 (1963)
47. Windhager, E. E., Giebisch, G., *Am. J. Physiol.*, **200,** 581–90 (1961)
48. Walker, A. M., Oliver, J., *Am. J. Physiol.*, **134,** 562–79 (1941)
49. Bennett, C. M., Clapp, J. R., Berliner, R. W., *Am. J. Physiol.*, **213,** 1254–62 (1967)
50. Gottschalk, C. W., Mylle, M., *Am. J. Physiol.*, **189,** 323–28 (1957)
51. Koch, K. M., Dume, Th., Krause, H. H., Ochwadt, B., *Pflüger's Arch. Ges. Physiol.*, **295,** 72–9 (1967)
52. Gertz, K. H., Mangos, J. A., Braun, G., Pagel, H. D., *Pflüger's Arch. Ges. Physiol.*, **288,** 369–74 (1966)
53. Maren, T. H., *Physiol. Rev.*, **47,** 595–781 (1967)
54. Clapp, J. R., Watson, J. F., Berliner, R. W., *Am. J. Physiol.*, **205,** 693–96 (1963)
55. Rector, F. C., Jr., Carter, N. W., Seldin, D. W., *J. Clin. Invest.*, **44,** 278–90 (1965)
56. Weinstein, S. W., *Am. J. Physiol.*, **214,** 222–27 (1968)
57. Bernstein, B. A., Clapp, J. R., *Am. J. Physiol.*, **214,** 251–57 (1968)
58. Meng, K., *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.*, **257,** 355–71 (1967)
59. Von Holzgreve, H., Loeschke, K., *Pflüger's Arch. Ges. Physiol.*, **294,** R 22–3 (1967)
60. Gottschalk, C. W., Lassiter, W. E., Mylle, M., *Am. J. Physiol.*, **198,** 581–85 (1960)
61. Bank, N., Aynedjian, H. S., *J. Clin. Invest.*, **46,** 95–102 (1967)
62. Malnic, G., Klose, R. M., Giebisch, G., *Am. J. Physiol.*, **206,** 674–86 (1964)
63. Malnic, G., Klose, R. M., Giebisch, G., *Am. J. Physiol.*, **211,** 529–47 (1966)
64. Giebisch, G., Malnic, G., Klose, R. M., Windhager, E. E., *Am. J. Physiol.*, **211,** 560–68 (1966)
65. Clapp, J. R., Robinson, R. R., *J. Clin. Invest.*, **46,** 1045–46 (1967)
66. Giebisch, G., *J. Cellular Comp. Physiol.*, **51,** 221–39 (1958)
67. Ullrich, K. J., *Biochem. Pharmacol.*, **9,** 181–89 (1962)
68. Ullrich, K. J., Twenty-third Intern. Congr. Physiol. Sci., *Excerpta Med. Congr.*, Ser. # 87, 53–66 (1965)
69. Thurau, K., *Ann. N.Y. Acad. Sci.*, **139,** Art. 2, 388–99 (1966)
70. Krause, H. H., Dume, Th., Koch, K. M., Ochwadt, B., *Pflüger's Arch. Ges. Physiol.*, **295,** 80–9 (1967)
71. Ullrich, K. J., Baumann, K., Loeschke, K., Rumrich, G., Stolte, H., *Ann. N.Y. Acad. Sci.*, **139,** Art. 2, 416–23 (1966)
72. Bennett, C. M., Brenner, B. M., Berliner, R. W., *J. Clin. Invest.*, **47,** 203–16 (1968)
73. Deetjen, P., *Ann. N.Y. Acad. Sci.*, **139,** Art. 2, 408–15 (1966)
74. Malnic, G., Vieira, F. L., Enokibara, H., *Nature*, **208,** 80–1 (1965)