RENAL PHARMACOLOGY[1]

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This is a selective review of literature published in 1966. In order to improve clarity of exposition and to facilitate interpretation, it was necessary also to include some older articles as well as a few published in early 1967. Some of the literature covered, especially in the various sections on renal transport, does not deal primarily with actions of drugs. It is considered because it has direct bearing on interpretation or understanding of results of pharmacological studies.

In many respects 1966 was a banner year for renal physiology and pharmacology. Several new and promising methods were introduced. Micropuncture and stop-flow, methods in vogue, were reappraised. The latest information on diuretics was collated and discussed at a symposium sponsored by the New York Academy of Sciences. Many excellent articles on reabsorption of sodium were published. These were by no means the only substantial accomplishments, but they do mark 1966 as "the year of the close look."

Advances in Methodology

Several new techniques or useful modifications of existing ones were introduced in 1966. Especially promising was a method for perfusing isolated segments of single rabbit nephrons. Its proponents, Burg et al., reported that perfused segments of proximal tubule remained viable for hours, reabsorbed fluid, and secreted *p*-aminohippurate (1). In a subsequent paper (2), they showed that vasopressin or cyclic 3′,5′-AMP added to the medium bathing the outside of the isolated, perfused collecting duct increased permeability to water but did not alter permeability to urea. Thus, as in amphibian membrane (3), there appeared to be separate barriers or sites for permeation of water and urea molecules. The method clearly offers a means toward solution of a large number of pharmacological problems, e.g. renal tubular handling of drugs, and mechanism and locus of action of inhibitors of active transport. Most advantageous is the fact that segments of the interior (i.e. those not available to micropuncturists) can be studied.

Another innovation of 1966 was the technique of retrograde intraluminal injection (RII) into the canine kidney (4). An outgrowth of stop-flow, the primary objective of the technique was to study the reabsorptive process. In

[1] The following abbreviations have been used: $U/_{P_{inulin}}$ *for* concentration of inulin in urine/concentration in plasma; $TF/_{P_{inulin}}$ *for* concentration of inulin in tubular fluid/concentration in plasma; PAH *for* *p*-amino hippuric acid; NMN *for* N-methyl nicotinamide.

order to reduce or eliminate for a time simultaneous occurrence of glomerular filtration and tubular secretion, substances were dissolved in solutions containing inulin and mannitol, and were injected under pressure through a ureteral catheter whose tip was lodged in the renal pelvis. After a suitable period of occlusion, fluid was re-collected fractionally as in stop-flow. Glucose injected in this manner was reabsorbed at the same place (presumably the proximal tubule) where *p*-aminohippurate injected intravenously was secreted. When administered by RII, the cysteine adduct of chlormerodrin moved into proximal tubular cells but its entry, unlike that of glucose, was inhibited by probenecid. Simple to perform and not requiring elaborate equipment, the RII technique should evoke interest in the relatively untrodden field of renal reabsorption of drugs. Other applications will be mentioned in a later section.

In addition to the methods discussed above, a few interesting variations of the stop-flow procedure were introduced or brought to fruition during the period covered by this review. One was the push-flow technique of Aukland & Kjekshus (5). In contradistinction to stop-flow, push-flow reduces the time during which composition of luminal urine can be altered by activity of tubular cells. Reduction is accomplished by infusing mannitol at a rapid rate. A push-flow pattern is the antithesis of a stop-flow pattern derived from distal segments. Sodium concentration rises and U/P inulin falls when contact time between tubules and urine is shortened abruptly. The chief advantage of push-flow is that ureteral occlusion with attendant distortion of intrarenal pressure relationships is avoided. The authors claim it is possible to estimate the magnitude of secretory and reabsorptive transport processes. Accordingly, they divided push-flow diagrams arbitrarily into four segments for which they calculated reabsorption of water. Such data have only limited value because each segment contains urine derived from different levels of a large number of nephrons. The technique, nevertheless, should be quite useful to the pharmacologist interested in comparing actions of drugs.

An uncertain feature of stop-flow is the proximal portion of the pattern. Since urine obtained largely from the proximal tubule [i.e. area of maximal secretion of *p*-aminohippurate (PAH)] passes through and can be changed by the rest of the nephron on its way out, it is virtually impossible to define the locus of action of a drug that alters the proximal part of the stop-flow curve. In order to deal with this problem, Gussin & Cafruny (6) modified the stop-flow procedure. One ureter was occluded and simultaneously ethacrynic acid was injected intravenously. Three minutes later, the second ureter was occluded. Their purpose was to confine the drug primarily to proximal portions of the nephron on one side but to allow it to pass into the entire nephron on the opposite side. This was possible because several investigators have shown that glomerular filtration decreases rapidly after ureteral occlusion during mannitol diuresis (7–9), and because ethacrynic acid is actively secreted by proximal tubular cells (10). Resultant elevation of the proximal limb of the stop-flow pattern from the side where the drug was confined, and

of the entire pattern from the other side, established the validity of using this modified procedure to study actions of drugs on the proximal portion of stop-flow patterns. The technique has also been used to investigate tubular secretion of drugs (4).

Ischemic stop-flow techniques, in which the renal artery and vein are clamped at the same time as or shortly after the ureter is occluded (11, 12), continue to be popular in Ireland. Most reassuring is the fact that data obtained by this technique are equivalent to those obtained by the parent method. Ashby et al. (13), unconvinced by results of the conventional procedure (14), used ischemic stop-flow to affirm the report that acetazolamide interferes with distal reabsorption of sodium. When they infused hypertonic saline, the distal minimum for sodium rose with increasing levels of sodium diuresis. Quite naturally, they questioned the validity of making comparisons between distal minima of consecutive occlusions if free-flow levels did not remain constant. In this view they do not distinguish between elevation of the distal minimum brought about by increasing sodium load and that which follows administration of a diuretic that does not increase load. In the latter situation it is reasonable to compare patterns. There is a direct correlation between height of the distal minimum and the rate of filtration of sodium (15).

No less valuable than methodological advances during 1966 were the articles of Orloff (16) and Rector et al. (17) on pitfalls in stop-flow and pitfalls in micropuncture. Orloff emphasized the advantages of stop-flow when it is applied to the study of substances transported at a single locus (e.g. PAH or glucose in the proximal tubule), but questioned its usefulness for studying substances transported at multiple loci (e.g. sodium). His chief arguments were that smearing of the concentration profile is inevitable and that, since all urine from the proximal part of the nephron must pass through distal parts before it can be collected, a so-called proximal effect of a drug can never be unequivocally defined. These are telling arguments and, undoubtedly, many of us have fallen into the pit, but the problems are not insurmountable. Smearing is not a serious problem when markers are used judiciously to delineate loci. Modified techniques, such as those just discussed, may provide evidence, though not proof, of proximal effects of drugs.

Those who have endowed micropuncture techniques with divine infallibility will be surprised to discover that they are beset with difficulties at least as great as those of stop-flow. Rector et al. (17) did not mince words about them. This group of experts cautioned against sampling of tubular fluid for estimation of distal tubular reabsorption, on the grounds that small alterations in proximal reabsorption may produce big changes in the volume of fluid entering the distal convolution. During periods of extreme diuresis, intratubular pressure rises and there is a greater chance of obtaining samples contaminated by retrograde flow from more distal portions of the nephron. They demonstrated that proximal and distal tubules dilate in response to pressure, and this results in an increase in transit time, fluid moving more slowly through larger lumens. Increased time of passage may enhance re-

absorption of sodium in the proximal tubule. Added to these problems are the usual errors of collection of tubular fluid and measurement of inulin which they estimate to be about 10 per cent. With an error of this magnitude at a low TF/P inulin of 1.5, per cent of calculated reabsorption ranges from 26 to 40. Thus an enormous over- or underestimate is possible. Needless to say, this important analysis should discourage adovcates of the closed-circuit approach to renal biology and strengthen our resolve not to be biased or wedded to any single technique.

Transport of Sodium in the Proximal Tubule

No single topic of renal phsyiology received as much attention during 1966 as studies on reabsorption of sodium and water in the proximal tubule. Contributions were so outstanding, they will undoubtedly alter many of the concepts of renal pharmacology as well as those of renal physiology.

Since 1961 the predominating opinion on transport of water and sodium chloride in the proximal tubule has been the one expressed by Giebisch (18). Briefly, this theory states that sodium diffuses passively into the proximal tubular cell but then is actively transported across the peritubular membrane; chloride follows sodium; the resultant osmotic force generated by movement of the two ions obligates reabsorption of water. Theoretically, either a change in rate of inward diffusion or in activity of the "sodium pump" will bring about a corresponding change in rate of reabsorption. Although they did not upset the one singular feature of this concept–the primacy or dominance of sodium transport, Rector and his co-workers (19–20) have argued for revision. They found that glomerulotubular balance, i.e. continued reabsorption of a constant fraction of the glomerular filtrate, continued when glomerular filtration rate was reduced by constricting the aorta, but did not continue when it was reduced by increasing the renal pelvic pressure. In the first condition, proximal tubular volume decreased but in the latter, volume expanded. Reabsorption increased in proportion to the square of the tubular radius, thus affirming the proposal of Gertz (21) that proximal tubular reabsorption might be related to tubular size. They concluded that it was not diffusion of sodium, but rather bulk flow of fluid through pores in the luminal membrane, that was the rate-limiting step in promixal tubular reabsorption. As bulk flow of tubular fluid into the cell increases, the rise in cytoplasmic concentration of sodium stimulates active outward transport of sodium. This is an ingenious explanation for the mechanism by which glomerulotubular balance is maintained, but it does not subvert the concept that humoral substances (endogenous or administered) may alter balance by influencing active transport directly. Hierholzer et al. (22) reported that the capacity of proximal tubular epithelium to transport sodium is impaired in adrenalectomized rats and that mineralocorticoids increase fractional reabsorption (23, 24). Morevoer, Rector et al. (25) have shown recently that saline inhibits intrinsic reabsorptive capacity of the proximal tubule. Thus, reabsorption of sodium appears to be a complex function of both physical and

chemical forces which together maintain glomerulotubular balance.

Many reports on the mechanism by which sodium loads suppress reabsorption in the proximal tubule were published in 1966. Interest in the topic was sparked by DeWardener et al. (26) and Mills et al. (27), who found that dogs infused with saline excreted large amounts of sodium even when glomerular filtration of the ion was not increased; that this augmentation of sodium excretion took place in denervated kidney and in isolated kidneys; and that salt-retaining steroids or vasopressin were not involved in the response. Thus, it appeared that some humoral substance, whose concentration was dependent on extracellular fluid volume, helped to control the rate of sodium excretion. These results were confirmed and extended (see 28–31), and finally, in 1965 Dirks et al. (32) showed that salt loads reduced fractional reabsorption in the proximal tubule of the dog. Watson (33) found that the magnitude of proximal depression was great enough to account for all of the extra sodium excreted.

Although there is as yet no decisive work on mechanism, reports of 1966 narrow possibilities. Levinsky (34) systematically eliminated the following possible mechanisms: (*a*) dilution of plasma proteins so that passive reabsorption of sodium diminishes; (*b*) release of a salt-losing hormone from the adrenal, liver, intestine, pituitary, or anterior part of the brain; (*c*) increased concentration of circulating angiotensin. Furthermore, an increase in filtered sodium brought about by procedures that elevate filtration rate without expanding extracellular volume had little effect on sodium excretion [Lindheimer (34)]. Earley & Friedler (35, 36) had suggested that sodium loads in part might inhibit tubular reabsorption of sodium in the ascending limb of the loop of Henle by increasing medullary blood flow (washout of medullary sodium retards movement of water out of descending limb). However, their views were altered after finding that in kidneys in which vascular dilation was maintained by intra-arterial infusion of acetylcholine or other dilators, angiotensin and norepinephrine depress reabsorption of sodium even though they simultaneously reduce glomerular filtration, renal plasma flow, and "non-cortical" plasma flow. They now believe that arterial pressure and renal vascular resistance play an important role in the regulation of sodium reabsorption (37–39). Shuster et al. (40) reported that rapid infusion of saline probably increased reabsorption in the ascending limb and that the combination of aortic clamping and saline infusion not only decreased reabsorption of sodium but also decreased total and non-cortical plasma flow. Cirksena et al. (41) found that the depression of proximal tubular reabsorption that follows saline loading could be prevented and even reversed during acute obstruction of the thoracic inferior vena cava. The effect was independent of changes in filtration rate or renal venous pressure. This finding can best be explained by assuming that a salt-losing factor is produced in greater amounts by normal dogs, but not those with caval constriction, during salt-loading. The possibility that release of a sodium-retaining factor is suppressed cannot be excluded. Earlier studies pointing to involvement of a

humoral substance have recently been supported by a report that sodium excretion increased in dogs that received blood from salt-loaded donors even though the recipient animals filtered less sodium (42). There are provocative data indicating that the autonomic nervous system modulates, at least in part, the natriuretic response to salt-loading. Several investigators (43–45) have shown that infusion of saline reduced sympathetic activity, and Gill et al. (46) found that adrenalectomized dogs with acute constriction of the thoracic inferior vena cava did respond to saline infusion in the usual way when ganglia were blocked with pentolinium. Thus, the importance of renal arteriolar resistance in the regulation of reabsorption appears to have been affirmed. Additional support for the Earley & Friedler suggestion comes from the observation that dogs with elevated blood pressure (due to infusion of metaraminol) excreted more sodium in response to salt-loading than did normotensive animals (47). However, this observation is not conclusive since effects of metaraminol alone were not studied in a sufficient number of animals, and it is possible that the drug may be natriuretic in some manner unrelated to its hypertensive action. Ben-Ishay & Dahl (48) did not find exaggerated natriuresis in salt-loaded rats with elevated blood pressure, but it is known that fractional reabsorption in the proximal tubule is not altered readily by administration of salt loads (49).

The following scheme brings together all foregoing information regarding the effects of salt-loading on sodium reabsorption and summarizes what workers in the field have accomplished: Salt-loading expands extracellular volume. This stimulates production and release of a natriuretic hormone at some unknown site or shuts down production of a salt-retaining hormone. Alternatively, expansion of extracellular volume lowers activity of the autonomic nervous system, thereby reducing renal arteriolar resistance. Although sodium reabsorption in the proximal tubule depends primarily on interrelationships between glomerular filtration rate, renal perfusion pressure, and vascular tone of post-glomerular capillaries, the humoral factor brought into play or excluded by administration of salt not only affects these interrelationships but also influences reabsorptive capacity of proximal tubular cells. The most definitive evidence in support of this latter point was recently published by Rector et al. (25), who found in rats that saline inhibited intrinsic reabsorptive capacity and reduced tubular size relative to any given level of glomerular filtration rate.

Renal Transport of Potassium

It is virtually impossible to increase rate of urinary excretion of sodium without concomitantly altering rate of excretion of potassium ion. Most diuretics force excretion of the ion, but some of the weaker ones reduce it and the mercurials, alone, exert either effect (on a net basis). The diverse actions of diuretics are undoubtedly related to the fact that potassium moves bidirectionally across renal epithelium. If it were possible to disentangle mechanisms and sites of transport, we should be able to appreciate more fully the

pharmacological effects of diuretic drugs. Knowledge of these mechanisms and sites derives largely from the experimental data that established the following points: (*a*) potassium is both secreted and reabsorbed (50, 51); (*b*) potassium exchanges for luminal sodium in the distal tubule (52, 53); (*c*) potassium is secreted at the same site where acidification of urine and secretion of ammonia occurs (54); (*d*) non-permeating anions increase excretion of potassium (55–57); (*e*) the ion is reabsorbed in the proximal tubule (58) against an electrochemical gradient (59, 60) and also is added to urine in lumens of collecting ducts (61); (*f*) maximal secretion of potassium can occur only when distal tubular fluid contains large amounts of sodium (62). Most available data affirm or support Berliner's model (63), in which potassium is reabsorbed virtually *in toto* in the proximal segments of the nephron and is secreted in the distal segments. However, there is also reabsorptive transport in distal segments (64, 65).

The elaborate microperfusion experiments of Malnic et al. (66) in 1966 added greatly to our knowledge of the subject. Their most important finding was that both proximal and distal tubular epithelium of the rat could establish concentrations of potassium significantly lower than those expected from measured electrical potentials. Thus, electrochemical equilibrium for potassium across the distal tubule did not develop because of active transfer of potassium out of the lumen. They emphasized that there was no need to assume any specific active secretion across the luminal membrane since direction and magnitude of the electrical gradient were adequate to effect net entry (66). If this interpretation is correct, then there must be two active forces regulating movement of potassium across the distal tubular cell–a peritubular pump forcing the ion into the cell from the blood side and a luminal pump forcing it in from the urinary side. Final reabsorption or secretion into urine would involve passive steps across single membranes that are actively transporting the ion in opposing directions.

Another clarifying paper was that of Watson (67), who used micropuncture methods to study fractional reabsorption of potassium in the proximal tubule of the dog during proximal tubular inhibition of sodium reabsorption produced by isotonic saline infusion, during stimulation of potassium secretion by potassium loading and administration of acetazolamide, and under conditions of reduced glomerular filtration. In no case was fractional reabsorption significantly modified, but inhibition of hydrogen ion secretion in the proximal tubule of animals that received acetazolamide with potassium infusion depressed sodium reabsorption (67). One may infer from this study that the increase in excretion of potassium brought about by diuretics is not the result, even in part, of failure of reabsorption in the proximal tubule, and that, as previously reported (68), acetazolamide blocks sodium reabsorption in the proximal tubule.

Diuretics

Site of Action in the Proximal Tubule.—The tubular site of action of diuretic agents has been and continues to be an arresting topic in renal

pharmacology. As each new technique or diuretic becomes available, there is a flurry of activity culminating in the publication of many "site of action" papers. Some have been excellent, but none has been definitive primarily because all methods used were indirect. It appeared that the only way to get answers was to use micropuncture methods. This was done in 1965 and 1966. Deetjen (69, 70) found a significant reduction in proximal tubular reabsorption in the rat after administration of furosemide, but only when glomerular filtration was 60 per cent or less of control rate. Malnic et al. (71) did not observe an unequivocal effect in the rat proximal tubule. Berliner et al. (68) and Dirks et al. (72) collected proximal fluid from dog tubule and then re-collected from the same site after administration of one of several diuretics. There was no reabsorptive deficit even when the most effective diuretics (ethacrynic acid, furosemide, chlormerodrin, hydrochlorothiazide) were used. Indeed, unless urinary losses were replaced with isotonic saline, reabsorption actually increased. Acetazolamide inhibited sodium reabsorption but not to a marked degree. These results were interpreted cautiously, the conclusion being that any direct effects of the diuretics on proximal tubular reabsorption in the dog must be counter-balanced through intervention of compensating local adjustments (e.g. tubular dilation) to the extent that there was no appreciable contribution to the final diuresis. The same problem was studied in the rat by Rector et al. (17, 25) who used free-flow micropuncture methods, in addition to the shrinking-drop technique of Gertz (73), to measure tubular volume and intrinsic reabsorptive capacity. Furosemide markedly inhibited reabsorptive capacity but did not suppress fractional reabsorption in the proximal tubule because of a restrictive increase in tubular volume; saline not only inhibited intrinsic reabsorptive capacity but also suppressed proximal reabsorption because of a permissive reduction in volume. That the limitation of reabsorptive capacity brought about by furosemide was the result of a direct effect on active transport of sodium may be inferred from the data of Ullrich et al. (74), who found that furosemide and chlorthalidone did not alter passive permeability to sodium or chloride. These diuretics inhibited active transport of sodium in both the proximal and the distal tubule. Furosemide had a greater effect in the proximal, and chlorthalidone in the distal convolution (74).

On the basis of the direct micropuncture studies cited above and other types of experiments to be discussed later, it is necessary to conclude that major diuretics exert pharmacological effects in the proximal tubule. The question is not whether such effects take place but rather whether they prevail over possible compensatory adjustments. There is no answer to the question, nor is there likely to be one in the near future. Absence of a net effect on proximal sodium reabsorption under conditions of micropuncture in which the kidney is dislocated, partially decapsulated, and stabbed does not prove that there will be no net effect under all conditions or that compensatory dilation will always oppose and match a reabsorptive deficit. The time course of events after administration of a diuretic has not been investigated in

micropuncture studies, and it is possible that net suppression of reabsorption occurs up to 15 to 20 minutes after injection until compensatory forces interfere. Ethacrynic acid, hydrochlorothiazide, and furosemide act quickly. Furthermore, the terminal third portion of the proximal tubule, as well as the entire proximal tubule of interior nephrons, is not accessible for sampling by micropuncture. Although these criticisms are not so strong as to lead us to discount the possibility that the proximal tubule adds no extra salt to the final urine when diuretic drugs are used, they reveal why cursory acceptance of conclusions derived from micropuncture studies alone should be discouraged. Unfortunately, clearance data are often difficult to interpret. Seldin et al. (75) reported that a massive dose of furosemide suppressed proximal reabsorption in hydropenic dogs, but they expressed doubt that this inhibitory effect occurs in circumstances where potent diuretics are commonly employed and suggested that the intense stimulus to proximal tubular reabsorption found in edematous states doubtlessly obliterates the inhibitory influence of the drug at this site. There is ample reason to reject their suggestion for, whatever the intense stimulus may be, a positive reabsorptive adjustment ultimately depends on dilation and increase in volume of the proximal tubule. Although the effect of diuretics on reabsorptive capacity may be neutralized in normal animals, one may argue that the effect is more likely to be expressed in edematous states in which little if any additional adjustment is possible. Although Gussin & Cafruny (6) found that ethacrynic acid inhibited proximal tubular reabsorption under conditions which prevented the drug from entering distal segments during stop-flow, their data and the earlier data of Beyer et al. (76) do not reveal whether the proximal action of the drug is expressed or overcome by compensatory dilation. Stop-flow, like the shrinking-drop method, only measures intrinsic reabsorptive capacity.

Effects on renal hemodynamics.—Barger (77) reported that sodium retention occurs in the dog when renal blood flow is largely distributed to inner cortical and outer medullary regions. Furosemide and ethacrynic acid both tended to normalize intrarenal distribution of flow.

Whereas the thiazides on occasion reduce PAH clearance and glomerular filtration rate (78), furosemide sometimes increases both (79, 80). For this reason, many investigators tacitly expected patients with low filtration rates to respond more readily to furosemide. Reubi (81) substantiated the point and, since inulin clearance increased transiently when the initial clearance was less than 50 ml/min, declared that a direct effect of the drug on glomerular filtration magnifies diuretic action in patients with severe renal disease. The actual increase in clearance, however, was quite small, ranging from about 2–18 ml/min. There was no indication of the duration of the effect or of the relation of the size of the natriuretic response to the change in clearance. In all probability the tubular effect of the drug was a more important factor than the vascular effect in governing the response. Consistent with Reubi's findings (81) were those of Hook et al. (82) in the dog. These workers reported that ethacrynic acid and furosemide reduced, but hydro-

chlorothiazide increased renal vascular resistance. Since mercurial diuretics can also reduce glomerular filtration rate (83) and increase renal vascular resistance (84), they postulated that an increase in renal blood flow underlies the frequent observation that patients refractory to thiazides or mercurials may respond to the newer agents. Their suggestion may be correct, especially if the rise in blood flow leads to expansion of cortical interstitial volume [see (37)]. Harvey (85) reported that acetylcholine infused into the renal artery of dogs lowered extraction but increased transport of PAH and NMN. Thus, the diuretic effect of the autonomic agent (86–88) is associated with generalized renal vasodilation. Perhaps the vascular actions of ethacrynic acid and furosemide do add significantly to the total diuretic response, but it remains to be seen whether therapeutic doses given orally actually improve renal blood flow. It comes as no surprise that these compounds are often more useful than thiazides or mercurials in patients with renal insufficiency, for they are more effective than thiazides under any conditions, and mercurials cannot be employed clinically in maximally effective doses.

Miscellaneous actions.—Mudge (89) reviewed literature dealing with the dependence of diuretic action of several drugs on conditions of acid-base balance. He pointed out that alterations in acid-base balance may produce a change in the underlying (diuretic) receptor or receptor mechanism, influence disposition of the diuretic agent, or bring about some change in the biological significance of the drug-receptor complex. Most published data [see (90–92)] support the view that effects of pH on activity of mercurials, azetazolamide, and methylated xanthines are due to a shift in the biological importance of the drug-receptor complex.

Talso et al. (93) found no changes in electrolyte content of skeletal muscle of rats that had received small amounts of a thiazide or chlorthalidone by mouth for a period of four weeks. They concluded that hypokalemia associated with oral diuretic therapy results from a redistribution of potassium stores rather than frank depletion. In spite of the fact that large numbers of animals were used (n = 20/group), only the group on chlorthalidone displayed a highly significant reduction in serum potassium and the reduction was rather small (from 5.35 to 4.87 meq/l). After finding that hydrochlorothiazide and dichlorphenamide given as a combination to hypertensive patients produced a marked decrease in total body potassium (94), they amended their original proposal.

Kessler (95) discussed the association between oxygen consumption and sodium reabsorption in the kidney. His hypothesis that oxidative metabolism energizes bulk sodium reabsorption without intermediation of ATP was bolstered by data demonstrating the fact that magnitude of sodium reabsorption is not necessarily influenced by procedures that cut renal ATP synthesis in half. This was noted when chloromerodrin was injected into a single renal artery. Renal ATP synthesis decreased by the same amount in both kidneys but diuresis occurred only on the injected side. The only situation in which changes in sodium reabsorption are not accompanied by equivalent

changes in oxygen consumption such that Na/O_2 remains constant is during osmotic diuresis. Kiil et al. (96) have demonstrated that mannitol in amounts which decrease net sodium reabsorption have no effect on renal oxygen consumption. In explanation, they suggested that the reduction in Na/O_2 was only an apparent one; recycling of sodium (i.e. passive movement into and active transport out of the proximal tubule) leads to an underestimate of actual transport. Knox et al. (97) confirmed the fact that Na/O_2 decreases during osmotic diuresis, but stated that the distal tubule and especially the ascending limb of Henle's loop, not the proximal tubule, reabsorbed much of the extra sodium that entered proximal urine by diffusion. Although cells of distal segments necessarily use more oxygen in the process, Na/O_2 of these cells should not differ substantially from that of proximal tubular cells. If this latter point is correct, a drug that blocks sodium reabsorption at distal sites only will not alter Na/O_2. Accordingly, they found that ethacrynic acid did not modify Na/O_2. However, the assumption that ethacrynic acid does not affect proximal tubular reabsorption is unwarranted, and it is conceivable that the change in Na/O_2 during osmotic diuresis reflects an inability of distal tubular cells to transport the same number of sodium equivalents per mole of oxygen consumed as proximal tubular cells.

Mercurial diuretics.—Several groups of investigators published data on mechanism of action of mercurials. Clarkson & Greenwood (98) reported that homogenates of renal tissue of rats injected with *p*-chloromercuribenzoate contained free mercuric ion and that all the mercury present was ionic during the period over which diuresis was observed. The same renal levels of mercuric ion produced diuresis whether released from *p*-chloromercuribenzoate or, as shown previously (99), from chlormerodrin. These data may be of great value when more information becomes available, but it is difficult at the present time to relate them to the mercuric ion hypothesis of Mudge & Weiner (100), because excretion of sodium chloride decreased and, even though all mercury present in renal tissue was ionic 15 minutes after injection of 3.7 mg Hg/kg as *p*-chloromercuribenzoate, there was no significant increase in urine flow at this dosage (98). Although the compound is not diuretic in the dog (101) when administered by conventional routes, Cafruny et al. (4) found that it did produce a small diuresis when it was given by retrograde injection. Since *p*-chloromercuribenzoate is a stable mercurial and apparently does not release mercuric ion in the dog (90), these data are in conflict with the massive amount of evidence supporting the mercuric ion hypothesis. The issue could be resolved if the compound were a weak agonist that triggered a short-lived response and then, as reported by Miller & Farah (102), acted as an antagonist. Such a response would not be detectible by conventional clearance determinations after intravenous administration. Littman et al. (103) found there was no consistent correlation between intrarenal distribution of ^{203}Hg-chlormerodrin and diuresis. This would be the expected result, if mercuric ion rather than organic mercurial were necessary for activity.

Published reports on site of action of mercurials included those of Levitt et al. (104) and Schmidt & Sullivan (105). Levitt andhis co-workers excluded the proximal tubule as a site of action largely on the grounds that mercurials did not increase C_{H_2O} or TC_{H_2O}, while mannitol, sulfate, urea, or hypertonic saline–substances known to interfere with proximal reabsorption–superimposed during mercurial diuresis were effective. Their data do not appear to be conclusive, for there was a distinct increase in C_{H_2O} which lasted for approximately 60 minutes after administration of a nontheopyhlline containing mercurial. Moreover, it is not clear why a substance that blocks distal reabsorption of sodium exclusively does not reduce C_{H_2O}. Schmidt & Sullivan (105) kept plasma sodium concentration at high levels in stop-flow experiments, and under these conditions mercurials elevated the distal minimum. These workers believe mercurials act on both proximal and distal tubules. Their evidence for distal inhibition of sodium reabsorption is good, but it is difficult to exclude the possibility that, as a consequence of inhibition in the proximal tubule, larger amounts of sodium were present in distal tubular urine at the time occlusion was performed. Otherwise, why should it be necessary to elevate plasma sodium to show an effect on the distal minimum when such an effect is readily apparent after administration of weaker agents, e.g. thiazides?

Thiazides and related drugs.—With few exceptions, most studies published in 1966 provided additional information about older observations. Reports of this type are often abstruse and require considerable discussion if they are to be reviewed properly. Valuable as these studies may be, space does not permit analysis, so most of them will merely be mentioned briefly.

Sullivan & Pirch (106) confirmed earlier reports that thiazides increase the height of the distal minimum in stop-flow studies (107, 108). There were many papers dealing with effects of thiazides on carbohydrate metabolism. Sitt et al. (109) clearly demonstrated that potentiation of the hyperglycemic response to diazoxide by hydrochlorothiazide was unrelated to the action of the diuretic on excretion of potassium. Henningsen & Benveniste (110) found that neither hydrochlorothiazide nor chlorthalidone produced any alteration in maximal rate of renal reabsorption of glucose. Fajans et al. (111) found that trichlormethiazide or diazoxide suppressed release of insulin from normal and abnormal islet tissue in man. Weller & Borondy (112) reported that chlorothiazide, not only decreased level of serum insulin-like activity, but also reduced the rate of utilization of glucose. Involvement of peripheral tissue was also observed by Barnett & Whitney (113), who reported that glucose uptake of rat hemidiaphragm was slowed by addition of chlorothiazide and diazoxide to incubation media.

A single intravenous injection of 5 mg hydrochlorothiazide/kg did not influence clearance of PAH or renal blood flow, but when the dose was doubled both were affected (114). Since clearance of PAH was depressed to a greater extent, the authors concluded that hydrochlorothiazide competitively blocked secretion of PAH. The ratio C PAH/C creatinine did not

change. Duggan (115) reported on accumulation of several thiazides in isolated renal tubules. His results clearly showed that four different agents were actively transported but that final tissue concentration was dependent on passive partition, a function of lipid solubility. This important study establishes a basis for the widely differing potencies of the various thiazides.

Travis et al. (116) studied the pharmacology of 2-benzenesulfonamido-1, 3, 4-thiadiazole-5-sulfonamide (CL 11, 366), a carbonic anhydrase inhibitor whose action is largely confined to the kidney. The drug produced maximal excretion of bicarbonate in doses as low as 0.5 mg. kg injected intramuscularly, but repeated administration of massive doses did not influence respiration. Highest concentrations of the drug were found in renal tissue and lung; little entered red cells. Eckstein et al. (117) reported that chronic administration of chlorothiazide reduced the constrictor response to infusions of norepinephrine in the dog. The effect was not altered by a ganglionic blocking agent; thus, it did not hinge on differences in vasomotor tone at the beginning of infusion.

Furosemide.—Other than those papers presented at a 1966 symposium on diuretic agents sponsored by the New York Academy of Sciences, most papers on furosemide in 1966 simply confirmed earlier literature covered in Annual Reviews for 1964 and 1965 (see 118, 119). Effectiveness of the drug in the presence of renal insufficiency was reaffirmed (120) and the fact that it may precipitate acute gouty arthritis (121), has a diabetogenic effect similar to that of thiazides (122), and lowers systolic pressure of hypertensives (123) was also reported again. There were two studies (124, 125) concerning its well-known effects on renal concentrating and diluting mechanisms. Calesnick et al. (126) gave S^{35} labeled furosemide to human volunteers and measured urinary excretion, fecal excretion, and salivary flow rates. About 80 per cent of an intravenous or intramuscular dose was excreted in urine during the first 24-hour period, but only 26 to 54 per cent was recovered after oral administration. Salivary flow increased but the saliva contained no detectable radioactivity. An extrarenal action of furosemide was mentioned by Baumung & Formanek (127), who found that furosemide increased serum sodium (from 110 to 140 meq/l) in nephrectomized rats. Hydrochlorothiazide in equieffective doses had no effect. Serum potassium was not altered. Furosemide did not reduce water or electrolyte content of muscle in normal subjects (128).

Ethacrynic acid.—In an effort to establish a biochemical basis for the diuretic action of ethacrynic acid, Duggan & Noll (129) used membranal ATPase preparations from the renal cortex of the rat, a species in which the drug is apparently ineffective. By incubating the tissue in deoxycholate, they were able to increase the potassium-dependent component of ATPase activity. Ethacrynic acid did not readily inhibit this component. These results are reminiscent of others (130–132) which also yielded circumstantial evidence for involvement of sulfhydryl enzymes in the mechanism of action of ethacrynic acid. Goldberg (133) reviewed literature on site and mode of ac-

tion of ethacrynic acid. He stressed two points: specific cellular mechanism of action is unknown; but site of action is predominantly, if not totally, the ascending limb of the loop of Henle. Flanagan & Ackerman (134) found that free water reabsorption often increased transiently in normal human volunteers, who excreted as much as 70 per cent of filtered sodium after intravenous administration of ethacrynic acid, and suggested that the proximal tubule is an additional site of action. Washington & Holland (135) noted that ethacrynic acid increases oxygen tension of urine after tension has been maximally reduced during saline or osmotic diuresis. They attributed this effect to drug-induced blockade of sodium transport in the ascending limb of the loop of Henle. Bourke et al. (136) studied effects of ethacrynic acid on excretion of urate and citrate in man. There was a transient increase in urate excretion at the peak of diuresis; this was followed by a sustained reduction in both excretion and clearance of urate for a period of four hours. The early increase in urate excretion was related to excretion of total solute. The increase occurred also in two of four individuals who were given hypertonic mannitol. Ethacrynic acid produced a marked reduction in excretion of citrate. The authors concluded that ethacrynic acid blocks tubular secretion of urate, and probably interferes with renal synthesis of citrate. Binder et al. (137) studied the actions of ethacrynic acid on transport across everted intestinal sacs. Movement of a variety of substances was impaired. These included water and sodium, amino acids, glucose, and uracil. An incidental finding was that probenecid, an inhibitor of renal transport of organic acids, interfered with intestinal transport of amino acids. Another extrarenal effect of ethacrynic acid was reported by Hoffman (138), who noted that the drug influenced sodium flux in human red blood cells. There were many reports on clinical responses to ethacrynic acid. Among these were papers dealing with ability to enhance bromuresis (139), effectiveness in the treatment of nephrogenic diabetes insipidus (140), absence of effect on glucose tolerance tests (141), pattern of diuretic action (142–145), and induction of acute transient hearing deficits in patients with severe renal impairment (146). Laragh et al. (147) summarized relevant clinical data of their large series of patients treated with ethacrynic acid or furosemide.

Other diuretics.—Although there were several publications in 1966 on other new diuretic agents (148–152), no unique or especially favorable property was mentioned. The renal effects of isosorbide, an orally effective osmotic diuretic, were described by Troncale et al. (153), and it was apparent that it might replace urea or mannitol in a number of clinical situations, but it has little effect on excretion of sodium. Liddle (154) directed attention to the point that triamterene is not an inhibitor of aldosterone. It fails to lower the sodium-potassium ratio of saliva as does spironolactone. Moreover, response to the combination of the two drugs greatly exceeds response to maximally effective doses of triamterene alone. Herman & Rado (155) discussed a case in which spironolactone produced a marked elevation of serum potassium (to

9.5 mEq/l). Baer (156) presented data on the pharmacology of amiloride (MK-870). This basic compound greatly enhanced the natriuretic action but antagonized the kaliuretic action of thiazides.

ADDENDUM

Since it has not been possible to review many valuable contributions to renal pharmacology for 1966, a partial listing of references is provided below for the reader who wishes to consult the original literature: the reninangiotensin system (157–167); aldosterone and glucocorticoids (168–171); autonomic agents (172); adenosine derivatives (173); ouabain (174); antidiuretic factors (175–178); ammonia (179–180); urea (181–182); excretion of organic compounds (183–187); clinical studies of diuretics (188–193).

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