MECHANISMS OF DRUG ABSORPTION AND EXCRETION[1,2,3,4]

THE RENAL EXCRETION OF DRUGS AND RELATED COMPOUNDS

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In view of adequate and detailed coverage in this area (2–11) and relatively slow accumulation of new information, it seems the most fruitful use of the allotted space would be to provide a brief recapitulation of several models for excretion of organic compounds and an analysis of recent findings in terms of these. These models are not mutually exclusive. They were developed for different compounds or sets of compounds; occasionally they represent results based on peculiarities of certain species. Although not yet demonstrated, it is possible that the elements of more than one model operate in the excretion of a given compound, i.e., a secreted and filtered compound may be reabsorbed by both nonionic diffusion and active transport.

Compounds will be considered without regard to therapeutic utility. The use of model compounds is frequently instructive and the demonstrated interrelations of the renal economies of endogenous substrates and drugs imply common pathways in their translocations.

Excretory Mechanisms

Filtration, active secretion, and reabsorption by nonionic diffusion.—The evidence for and examples of this mechanism which seems to have wide applicability have been summarized (10, 12). In this scheme, a wide variety of organic acids are filtered, and, in addition, are secreted by the tubular mechanism for organic acids [the hippurate or *p*-aminohippurate (PAH)

[1] The survey of the literature pertaining to this review was concluded in June 1966.

[2] The following abbreviations will be used: PAH (*p*-aminohippurate); U/P (urine concentration/plasma concentration); T_m (tubular maximum).

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[4] The term active transport will be used rather loosely. It will usually mean apparent uphill transport. In the case of reabsorbed substances it is difficult to apply even this definition. For example, urate is often said to be reabsorbed by active transport since reabsorption is rate-limited (1) and can be inhibited by pharmacological agents. However, its reabsorption could be via carrier-mediated diffusion and display these same characteristics. It would be necessary to show urine concentration /plasma concentration (U/P) ratios at the site of transport lower than that attributable to pH partition and potential to define this transport as active. Since this information is generally not available for organic compounds and repetition of qualifying phrases is cumbersome, I will refer to apparently specifically mediated reabsorption as "active."

mechanism]. The compounds are reabsorbed from the tubule by nonionic diffusion. This reabsorption ranges from insignificant levels to practically complete reabsorption depending on experimental conditions and the physical properties of the compounds. Reabsorption is favored by low urinary pH which increases the proportion of un-ionized molecules and by low rates of urine flow. The latter results in high U/P ratios which increase reabsorption proportionately. Stronger acids are reabsorbed less readily than weaker acids. High solubility of the un-ionized acid in lipid solvents (and by inference the lipid-like cell membranes) makes for high rates of absorption.

Despopoulos (11) disagrees with the notion that weak acids whose secretion is seen only with alkaline urine are secreted by the hippurate mechanism. However, it is clear that these compounds are secreted since their apparent secretion cannot be explained by nonionic diffusion (13, 14) and all other known physiological factors favor net reabsorption. The secretion of many of these is suppressed by the administration of PAH or hippurate (10, 12) making secretion via the hippurate mechanism an attractive hypothesis.

A similar mechanism has been described for organic base excretion (15). The secretory system is analogous to, but separate from, the mechanism for organic anions and the effect of urinary pH change is opposite in sign. It should be noted that the large pH difference attainable between acid urine and plasma theoretically allows for secretion to occur by nonionic diffusion alone. However, in several cases nonionic diffusion does not approach equilibrium with sufficient rapidity to account for observed excretion rates.

Active secretion and active reabsorption in the same tubular segment.—Kinter and his colleagues found that PAH and iodopyracet (Diodrast) are secreted by the tubules of some specimens of *Necturus*, reabsorbed by the tubules of others (16), and that translocation in either direction occurs in the proximal tubule (17). Tanner & Kinter found that reabsorption is not via nonionic diffusion (it is not sensitive to changes in urinary pH) and that it is probably active transport (18). Octanoate administration converts net reabsorption to net secretion *in vivo*, and in perfused kidneys where the control state is always net reabsorption. Both secretion and reabsorption can be inhibited by competitors for transport. On the basis of these and other findings, Tanner & Kinter proposed a convincing model for PAH transport in *Necturus*. Cells of the proximal tubule have two active transport mechanisms, one at the luminal and the other at the antiluminal membrane. Both pumps, which operate simultaneously, are oriented to move material into the cell from which it leaves by diffusion. Depending on which pump is more effective, net secretion or net reabsorption occurs. It is postulated that variations in the level of a blood-borne substance, which blocks the reabsorptive pump selectively, determine the direction of transport. Octanoate and other fatty acids mimic this substance.

A similar scheme was proposed by Cohen & Wittmann (19) for the transport of α-ketoglutarate. The latter is normally reabsorbed by a process limited by a maximal rate (T_m). However, net renal uptake of α-ketoglutarate can

exceed the quantity reabsorbed, thus the compound must also enter renal cells from the antiluminal side. Probenecid blocks the latter process. Stop-flow experiments identified the proximal tubule as the site of reabsorption and it was inferred that this is also the site of peritubular entrance since α-ketoglutarate blocks PAH uptake in this segment. The impaired reabsorption attending alkalosis is attributed to the accumulation in plasma of competing substrates for reabsorption. The evidence for this model is not so complete as that for PAH in *Necturus*, this being largely the result of the additional complication of intrarenal metabolism of α-ketoglutarate. It is still possible that the effect of probenecid on the renal uptake of the substrate is an indirect consequence of inhibition of α-ketoglutarate dissimilation. Most of the experimental findings reported in this study have been confirmed by Balagura & Pitts (20). These investigators are inclined to the view that intracellular pH determines the magnitudes of reabsorption and antiluminal transport. In a related study, Selleck & Cohen (21) observed that liver and kidney, the two organs in which transport mechanisms for foreign organic anions are most highly developed, are also the two main sites of α-ketoglutaric acid uptake from blood. They postulate that the organic anion mechanisms are normally primarily involved in the uptake of Krebs cycle intermediates.

Cohen & Prout (22, 23) confirmed earlier observations that another Krebs cycle intermediate, citrate, is reabsorbed in the proximal tubule. Their experiments with labeled citrate suggested that the luminal membrane allows movement of citrate into and out of proximal cells, influx predominating. Succinate, which inhibits citrate reabsorption, interferes with movement in both directions.

A third example of simultaneous active secretion and active reabsorption in the same nephron segment comes from stop-flow studies of urate excretion in the rabbit (24). In free-flow periods most rabbits demonstrated net reabsorption; a few, net secretion. On stop-flow all of the secretors and most of the reabsorbers yielded patterns of proximal tubular secretion. No evidence for transport in either direction was obtained for the distal nephron. Some of the reabsorbers produced a stop-flow pattern indicating proximal reabsorption. The results suggested bidirectional active transport with either process capable of predominating. Ureteral occlusion, for unknown reasons, favors the secretory component. Reabsorption seems more sensitive to the action of ouabain since the latter enhanced the proximal secretory peak (25).

Active secretion and active reabsorption in different tubular segments.—This model was suggested by Gutman & Yü (26) for the excretion of uric acid in man and mongrel dog. Bidirectional active transport is suggested by the observation that some uricosuric agents, e.g., salicylate (27), have paradoxical effects. At low doses they inhibit, and at high doses, enhance urate excretion. This was interpreted as the sequential inhibition of a sensitive secretory mechanism for urate and a more resistant reabsorptive mechanism. In some species, Dalmatian dog (28, 29), chicken (30), and rabbit (24, 31),

the secretory system is probably a proximal tubular function which seems similar to the hippurate system. In the mongrel dog and presumably in man, reabsorption occurs in the proximal tubule (28, 29). Under certain conditions, net tubular secretion can, to a small and variable degree, be induced (28, 32, 33). In stop-flow experiments under these conditions no proximal secretory peak is seen. In one series (28), in addition to the usual proximal reabsorptive dip, a distinct distal secretory peak was observed. This was not seen in other laboratories (29, 34). However, the notion of some sort of distal transtubular movement of urate in the mongrel dog received strong support from a study by Davis et al. (35). They injected urate-C^{14} 15 seconds before the release of occlusion in stop-flow experiments and found a distinct distal peak of radioactivity. This peak was eliminated by pretreatment with pyrazinamide which is known to dramatically decrease urate clearances in man (36, 37) and somewhat less so in the dog (38).

Distal secretion in man and dog would help explain some anomalies in urate clearance such as the paradoxical effects of uricosuric agents, and the action of pyrazinamide and its metabolites. These latter substances are much more effective inhibitors of urate than of PAH excretion (36), suggesting separate sites of action.

Pyrazinamide is hydrolyzed to pyrazinoic acid (36, 39). Preliminary evidence (40) indicates that about half the material in urine (dog and man) is pyrazinoic acid and most of the remainder 5-hydroxy-2-pyrazinoic acid. The clearance of pyrazinamide is low due to passive reabsorption. Pyrazinoic acid is reabsorbed by a rate-limited proximal tubular mechanism. The oxidized metabolite is secreted by a probenecid- and PAH-sensitive mechanism. Further studies may explain the relationship between these substances and uric acid.

According to the scheme of proximal reabsorption and distal secretion of urate in mongrel dog and man, the action of uricosuric agents is thought to result from (competitive?) inhibition of proximal reabsorption. Interestingly, many uricosuric agents are themselves secreted in the proximal tubule, e.g., salicylate, probenecid, sulfinpyrazone, iodopyracet. This does not preclude simultaneous reabsorption by the urate mechanism as long as it is smaller than the secretory process for these compounds. Such a scheme might explain observations suggesting that the amount of uricosuric agent in tubular fluid rather than in plasma determines the magnitude of effect. For example, salicylate is more potent when urine is alkaline and its excretion high (27). If salicylate competes with urate for reabsorption, drug lost from tubular fluid by nonionic diffusion is lost from the site of competition. The correlation between uricosuric activity and excretion rate among the analogues of phenylbutazone (41) might be similarly explained.

A clear example of proximal reabsorption and distal secretion is provided by ascorbic acid. Previously, the clearance of this substance was described as the resultant of glomerular filtration and active reabsorption [summary in (42)]. Kleit & co-workers (43) found that under conditions in which distal

Na^+-K^+ exchange is stimulated, and especially with alkuria, the clearance of ascorbic acid exceeds filtration rate. Under optimal secretory conditions, the stop-flow pattern indicates net proximal reabsorption and net distal secretion. The mechanisms by which procedures enhancing distal secretion operate are obscure. The physical properties of ascorbic acid argue against nonionic diffusion playing a role.

Filtration and active reabsorption.—This is the classical scheme for the handling of glucose and amino acids. Most substances known to be actively reabsorbed are endogenous compounds or close analogues. Young & Edwards (44, 45) characterized the renal handling of *l*-methylDOPA as filtration and active proximal reabsorption via one of the amino acid mechanisms. This agent causes a mixed aminoaciduria (44) attributable to competitive inhibition.

The reabsorptive system for bile salts is proximal tubular active transport (46). It was suggested that bile salts also participate in the renal acid secretory mechanism since bile salts inhibit the secretion of other acids (47) and share a similar hepatic secretory system with these compounds (48).

Filtration and active secretion.—This is the classical scheme for the renal excretion of substances such as phenol red, PAH, and tetraethylammonium ion. It can be considered a special case of the first scheme discussed above in which nonionic diffusion is reduced to insignificant levels by the physical properties of the compound under consideration. Evidence suggesting a small degree of reabsorption of the ionized fraction of some highly polar compounds has been reviewed (10).

Filtration and reabsorption by nonionic diffusion.—The best examples of this scheme are still barbital (49), phenobarbital (50), and 5,5-dimethyl-2,4-oxazolidinedione (51). Although the barbiturates are specific inhibitors of the PAH secretory mechanism (52), there is no evidence that they are themselves secreted. Probenecid has no effect on clearance (50).

Secretion by nonionic diffusion.—As indicated earlier, this process is theoretically possible for organic bases although many of the compounds studied require active secretory transport for the observed high rates of excretion. Neutral red may be an exception (53). At least it is possible to demonstrate secretion when the tubules are poisoned with urethane or cyanide. Whether secretion can be partially inhibited by competing bases (indicating an additional active component) has not been established.

Filtration and passive reabsorption not involving nonionic diffusion.—The most likely example of this is the mechanism for the excretion of urea. Rabinowitz (54) compared the excretion of urea with that of methylurea and acetamide at various plasma levels and rates of urine flow. He reaffirmed that urea is reabsorbed by passive diffusion. The relationship between oil-water partition coefficient and rate of reabsorption of the three substances is thought to reflect nonionic diffusion in the proximal tubule. Rabinowitz feels that in the distal nephron diffusion is through aqueous pores. This contention is supported by the action of an antidiuretic hormone to increase the perme-

ability of the distal system to urea (55, 56). Evidence for active components in the transport of urea into (57) and out of (58) the nephron continues to accumulate.

Apparent secretion of metabolites formed in kidney.—Ginsburg (59) found that arsenate is reabsorbed in the proximal tubule of dogs by a mechanism which is inhibited by elevated plasma phosphate or glucose levels, or by diuresis. On the other hand, the clearance of arsenite formed from arsenate in the body exceeds the glomerular filtration rate (GFR). The apparent secretion of arsenite occurs in the proximal tubule and its magnitude is decreased by interventions which decrease arsenate reabsorption. When arsenate is infused, the concentration of arsenite in renal venous plasma exceeds that in arterial plasma. When arsenite itself is infused, its clearance is less than filtration rate. The secretion of arsenite observed during arsenate infusion is probably the result of intrarenal formation of arsenite with subsequent diffusion into tubular fluid (as well as plasma).

The above mechanism is analogous to the renal mechanism for ammonia where clearance greatly exceeds filtration rate and most of the material in urine is formed in the tubular cells. Net transtubular secretion of ammonia can occur when this substance is infused (60), a case of secretion by nonionic diffusion.

Decreased secretion or apparent absence of secretion caused by renal metabolism.—Histamine is secreted in several species. In man, the kidney avidly extracts histamine from blood, whole blood extraction being 0.7–0.8 times the renal blood flow (61). However, only 10 per cent of this extracted material appears in the urine as histamine, the remainder is metabolized. In the avian kidney, serotonin secretion is seen only when its metabolism is blocked (62).

Component Systems

The secretory systems.—Although the relationship of plasma protein binding to pharmacological activity receives active attention (63, 64) there are no recent studies on the influence of such binding on tubular secretion. Because of the persistence (11) of an older, erroneous speculation on this subject, a brief discussion is warranted. Beyer (65) noted an inverse correlation between number and size of alkyl substituents and magnitude of clearance with a series of compounds related to probenecid. Weiner et al. (66) pointed out the inverse correlation between lipid solubility and clearance, and attributed the differences in clearance to differences in the magnitude of nonionic diffusion. Despopoulos (11) has taken issue with this on the basis that an inverse correlation also exists between clearance and extent of protein binding.

Quantitative considerations preclude the latter as a major determinant. At plasma concentrations of 20 mg per 100 ml, the various compounds have clearances ranging from 0.01 to approximately 1.3 times the filtration rate (without correction for binding). At this plasma concentration, unbound drug varies from 26 per cent for the compound with the lowest clearance to

95 per cent for the compound with the highest clearance (65). If protein binding of the substance with the lowest clearance were reduced to the lowest level in this series and the magnitude of secretion as well as filtration increased proportionately [this is the maximal increase (67, 68)], clearance would go up to only 0.04 of the filtration rate. Similarly, the change in magnitude (and direction) of net transtubular movement induced by alkalosis (67) cannot be attributed to changes in extent of protein binding.

A reasonable interpretation of available information is that with compounds having a high relative affinity for the transport mechanism, protein binding has little influence on clearance, while with compounds with lower affinity, clearance will be considerably influenced by the magnitude of binding. This is in contrast to hepatic secretion where highly bound substances seem readily transferable directly to the secreting cells (69).

The inverse correlation between plasma protein binding and net secretion of a series of sulfonphthalein dyes (70) is difficult to interpret because of tubular storage (71) and simultaneous reabsorption (72). The evidence for the interaction of urate and plasma proteins (73), which has important implications in the pathogenesis of gout, is difficult to reconcile with other work suggesting complete ultrafilterability (1).

It is commonly accepted that there are two separate major secretory paths for organic ions, the organic acid or anion mechanism and the organic base or cation mechanism. The reasons for regarding these as separate have been summarized (74). The characterization of these mechanisms in terms of the net charge of their substrates may be incorrect. Urea (uncharged) (75) is secreted by a probenecid- and PAH-sensitive mechanism in the frog, presumably the anion mechanism. There are some examples of substances which may be transported by both mechanisms. Creatinine secretion in the dog (76) is sensitive to inhibitors of both the acid and base systems. In male rats only inhibition by participants in the acid system has been shown to date (77). Rennick & Pryor (78) have shown that norepinephrine and DOPAmine are secreted by the avain tubule by mechanisms sensitive to both inhibitors of the anion and the cation systems. Interestingly, the secretion of epinephrine is not depressed by an inhibitor of the base system, cyanine 863, but is sensitive to inhibitors of the organic acid mechanism (79). Braun (80) has demonstrated pH-dependent excretion of epinephrine in dogs and rats. He suggested that epinephrine is subject to filtration, secretion, and nonionic reabsorption. Active secretion was not demonstrated in this study. Jones & Blake had previously shown that epinephrine clearance could exceed filtration rate in dogs (81).

Despopoulos (11) provided a noteworthy summary of the transport characteristics *in vivo*, *in vitro*, or in both of some 150 organic acids. He concluded that a compound must have a net negative charge to interact with the transport system and an additional carbonyl or sulfonyl oxygen which, by virtue of participation in hydrogen bond formation, activates the system. The interatomic distances of these functional groups fall into two classes: (*a*) a

class corresponding to the dimensions of the folded hippurate side-chain, and (*b*) a class corresponding to the extended hippurate side-chain. Although many compounds conform to this scheme there are some notable exceptions, e.g., iodopyracet, which indicate that the proposed structures are not absolute requirements. Some acids whose structures do not conform and whose excretion is pH-dependent have been excluded from consideration on the basis of criteria which this reviewer considers unjustifiably restrictive.

The kinetics of organic ion secretion *in vivo* (82) or uptake *in vitro* (83–85) seem to conform to the Michaelis-Menten scheme, and it has been generally accepted (with the usual reservations concerning complex systems) that tubular maxima are equivalent to the V_{max} of enzymology. However, microperfusion experiments in rats demonstrated that the influx of PAH into proximal tubular fluid is limited by a maximal concentration in that fluid (86). The results cannot be explained by simultaneous reabsorption. Accordingly, the authors propose that T_m is a function of this concentration and the volume of fluid traversing the proximal tubule per unit time. A similar phenomenon was encountered in a study of intraluminal chlorphenol red accumulation in isolated flounder tubules (87). The rate of accumulation was related to concentration of dye in the medium in a manner consistent with Michaelis-Menten kinetics. However, at a given dye concentration in the medium, uptake was linear until a critical luminal concentration was reached, at which point further accumulation ceased abruptly. Neither the shape of this uptake curve nor the experimentally determined values for influx and outflux are consistent with this phenomenon being the resultant of a constant pump activity with a concentration-dependent leak.

The inhibition of organic acid transport by 2,4-dinitrophenol has been attributed to its ability to uncouple oxidative phosphorylation (88). However, the closely related chemical, picric acid, has a similar effect on transport but is not an uncoupler (88). Moreover, dinitrophenol inhibits PAH transport *in vivo* without the expected saluresis or glucosuria (89) attending a general deficit in energy transformation. The recent study by Huang & Lin (90) may provide an explanation. They showed that inhibition of PAH transport by dinitrophenol in isolated rabbit tubules followed kinetics consistent with competition. In addition, they demonstrated the transport of dinitrophenol itself. It seems possible that under the conditions of *in vivo* experiments, dinitrophenol administration results in inefficient, but not grossly insufficient, energy transformation, and the relatively specific inhibition of PAH transport is the result of competition. Similar considerations may apply for fluoroacetate which is more effective against the organic acid than the organic base system *in vivo* (91).

Several studies demonstrated inhibition of renal transport of organic substances by cardiac glycosides [e.g. (92, 93)]. This is generally attributed to inhibition of the Na-K-activated ATPase. The notion is supported by the demonstration that PAH (and glucose) transport in perfused frog kidney depends on the sodium concentration in perfusion fluid (94).

The increase in PAH T_m during alkalosis has been attributed to the increase in blood lactate accompanying alkalosis (95). Transport of PAH is almost entirely destroyed by one hour of renal ischemia (96). The damage is reversible. Angiotensin has no effect on T_m PAH (or T_m glucose) (97). Mercaptomerin which reduces T_m PAH by 55 per cent in man has no such effect in the dog and actually reverses the reduction in T_m accompanying prolonged pentobarbital anesthesia (98). The mechanism is obscure. Post hemorrhagic hypotension as well as severe dehydration are accompanied by large decreases in T_m PAH approximately proportional to the accompanying reduction in plasma flow but not as severe as the reduction in GFR (99). Under these circumstances, extraction ratios can be normal if plasma PAH is sufficiently low. The finding that renal cortical slices from thyroidectomized animals have a decreased ability to concentrate PAH has been confirmed (100); this procedure has no effect on the uptake of tetraethylammonium ion. Slices from animals subjected to chronic thyroxine administration have decreased ability to concentrate both PAH and tetraethylammonium (100). Low concentrations of L-thyroxine and various related iodinated acids inhibit PAH uptake when added *in vitro* (101). It was suggested that this represented competitive inhibition.

In the past few years considerable attention has been given to the behavior of the secretory system *in vitro* and its relationship to events *in vivo*. *In vitro* preparations of mammalian kidney usually (102) but not always (103) lack the ability to affect intraluminal anion accumulation. This is in contrast to preparations from certain fish where either intraluminal or intracellular accumulation can be regularly observed depending on the ionic environment (71). These and other considerations led to the notion that the mammalian tubule can only pump organic anions across the peritubular membrane, giving a high intracellular concentration which allows diffusion into the lumen (104). Subsequent work made it necessary to postulate that movement into the lumen is specifically mediated (105). Now it seems likely that a concentrating mechanism is operating across the luminal membrane of mammalian proximal tubular cells. Deetjen & Sonnenberg (86) observed practically no loss of PAH when they perfused proximal tubules of rat kidney *in situ* with solutions containing PAH. Since the total volume of the surrounding cells is at least as large as that of the lumen, appreciable loss of PAH into the cellular volume should have occurred were the luminal membrane permeable to PAH even if the peritubular membrane was effectively impermeable. Since the luminal membrane allows PAH to enter the lumen, it is necessary to postulate a concentrative step to prevent net reabsorption through the liminal membrane in these experiments. The failure to observe consistently the operation of this concentrating mechanism *in vitro* requires explanation. It may be, as Beyer et al. (103) implied, that this system is extremely sensitive to injury.

When the reciprocal of the steady-state concentration of PAH or other compound in renal slices or tubules is plotted against the reciprocal of me-

dium concentration, the data of several workers [e.g. (83, 90)] conform to the linear Lineweaver-Burke relationship. This is explained by the recent demonstrations that outflux is largely a first order process (87, 106–108). Thus in the steady-state: influx = outflux = K_{out} C, where K_{out} is the first order constant and C is the concentration in tissue. Consequently, the steady-state concentration is directly proportional to influx. Possible complications arising from diffusion of material through slices (109) have not been evaluated.

Considerable effort has been expended in attempts to elucidate the mechanism of efflux from *in vitro* preparations of kidney tissue previously loaded with transported substrate. Earlier references are given by Kinter & Cline (110) and Farah et al. (106). Kinter & Cline (110), using the goldfish kidney, observed that upon transfer of tissue exposed to I^{131}-iodopyracet to a iodopyracet-free medium, most of the previously accumulated radioactivity disappeared from the tissue within 60 minutes. The rate of efflux was accelerated by metabolic inhibitors, e.g., cyanide, and this was attributed to inhibition of simultaneous active recapture of iodopyracet from the medium. Competitive inhibitors of active uptake of iodopyracet had a biphasic effect on runout. At low concentrations, these substances accelerated loss of iodopyracet from the tissue. At higher concentrations of inhibitors, the rate of runout returned to or below control levels. These results were interpreted in terms of carrier-mediated counter transport where such a biphasic response is an expected phenomenon (111).

Farah et al. (106) reported a similar study using slices of dog renal cortex and PAH under conditions which reduce recapture of material. Cyanide and azide accelerated efflux and competitive inhibitors gave the biphasic response. The increased rate of efflux produced by low concentrations of probenecid could not be demonstrated in the presence of cyanide. The carrier-mediated counter transport hypothesis would predict the opposite. Farah et al. proposed a two part hypothesis based on an earlier model (112). According to this, most of the PAH in the cell is trapped or bound. This trapping is specific, requires metabolic energy, and is therefore susceptible to inhibition by competitive inhibitors as well as metabolic poisons which consequently accelerate efflux. Exit from the cell is via a carrier mechanism which does not have the property of counter transport but which is susceptible to competitive inhibition, which results in the ability of competitive inhibitors to slow efflux when present in high concentrations. Unfortunately, it has not been possible to obtain direct evidence for the trapping or binding phenomenon (106, 112). Recently, Ross & Farah (108) have presented additional evidence against the operation of counter transport in this system. They were unable to demonstrate enhanced uptake of radioactive PAH (or N-methylnicotinamide) by slices preloaded with nonradioactive substrate. Counter transport should operate in both directions.

Interestingly, in the fish tubule (goldfish and flounder) the biphasic effects of competitive inhibitors on runout are not abolished by cyanide (87,

110). This tends to support the notion of counter transport in these species. However, Kinter (87) subjected this idea to critical tests and concluded that even in these species, counter transport does not account for the observed phenomena. The hypothesis of Farah et al. (106) for events in the dog slice does not seem applicable to tissue from fish. The accelerated efflux induced by competitive inhibitors is seen in the absence of oxidative metabolism and thus cannot be the result of competitive inhibition of an energy requiring trapping mechanism.

The biphasic response to competitive inhibitors has been observed in isolated rabbit tubules (90). Efflux of urate from rabbit renal slices is similar but not identical to that described for PAH (113). Efflux of organic bases from renal slices is not accelerated by competitive inhibitors, but the decrement in rate is seen with high concentrations of inhibitor (108).

Reabsorption.—Acetazolamide, a carbonic anhydrase inhibitor, has two effects on the excretion of salicylate and presumably other weak acids (13). When urine is initially acid, salicylate excretion is small and acetazolamide administration enhances excretion by urinary alkalinization. On the other hand, when the urine is initially alkaline and salicylate secretion is observed, acetazolamide inhibits salicylate excretion. This was attributed to competitive inhibition of salicylate secretion, since: (*a*) acetazolamide is secreted by a probenecid-sensitive mechanism as is salicylate, and (*b*) a closely related analogue of acetazolamide devoid of carbonic anhydrase activity also inhibited salicylate secretion. It now seems likely that an additional property of acetazolamide also contributes to the decrement in salicylate excretion.

Carbonic anhydrase inhibitors cause a fall in the pH of proximal (but not distal) tubular fluid of rats excreting alkaline urine (114). This is attributable to inhibition of carbonic anhydrase which has access to tubular contents and normally prevents the "disequilibrium pH" resulting from the slow, uncatalyzed rate of carbonic acid dehydration (115). The fall in pH induced by carbonic anhydrase inhibition can accelerate nonionic reabsorption of salicylate. The relative contributions of this fall in proximal pH and competitive inhibition to the decrease in salicylate excretion cannot be assessed at present. The increased acidity of proximal fluid after carbonic anhydrase inhibition may explain the finding that acetazolamide is less effective than bicarbonate in enhancing phenobarbital excretion (50). Presumably, acetazolamide cannot be a competitive inhibitor in this circumstance since phenobarbital is not secreted. This property of carbonic anhydrase inhibitors may have important bearing on their relation to the excretion of endogenous organic acids (116).

A study of diffusion of organic acids through mucosae of toad bladders at various pH values (117) has implications for renal pharmacology. Not all of the substances studied showed ideal behavior, i.e., rate of transmucosal movement proportional to the concentration of the nonionized fraction. This might be the result of some sort of decrease in chemical activity such as

micelle formation, a direct pH-induced change in the properties of the membrane, or saturation of the lipid in the membrane. Loss of lipid-soluble substances from mammalian bladders has been studied by Borzelleca (118).

The lack of sensitivity of PAH clearance to pH changes at low urine flows and low plasma levels has been confirmed (119). This result, which is indicative of little or no nonionic reabsorption, is consistent with the low lipid solubility of PAH. Little PAH is lost from rat proximal tubules perfused with solutions containing this substance (86, 120). Results were similar with iodohippurate in perfused tubules of salamanders (121).

In the microperfusion study by Sonnenberg and collaborators (120), five other acids were studied along with PAH. The polar compounds, uric acid and sulfaurea, were similar to PAH in that they were poorly absorbed. If anything, absorption was greater from alkaline perfusion fluid than from acid. The two lipid-soluble substances, phenobarbital and sulfamerazine, were readily absorbed and absorption was more rapid from acid perfusion fluids. The behavior of acetylsulfamerazine was unique. It was poorly absorbed, but unlike PAH, absorption was slightly greater from acid solution. Acetylsulfamerazine is a secreted substance which has appreciable lipid solubility. On this basis, Sonnenberg et al. imply that secreted substances cannot be absorbed by nonionic diffusion. The excretion of acetylsulfamerazine is much less pH-dependent (122) than is the excretion of phenobarbital (49) which showed only a twofold variation of absorption in this test system. Thus, it seems a poor choice with which to test the hypothesis.

Knoefel (123) suggested that the failure to observe active accumulation of lipid-soluble substances by renal tissue *in vitro* may be the result of a rapid leak of these substances through the containing membranes. Despopoulos & Segerfeldt (124) studied the runout of a variety of anions from kidney slices under anaerobic conditions. The rates of efflux were similar for all compounds and the authors concluded that the physical properties of the compounds have little relevance to their rate of efflux from renal tissue *in vivo* or *in vitro*. However, the experimental design (high concentration of substrate during the loading phase) minimized the proportion of compound in the compartment into which active transport occurred and therefore exaggerated the multicompartmental nature of the tissue. Since different compounds have different relative distributions in the various compartments and some are largely protein-bound in renal tissue, these conclusions seem tenuous.

Gekle et al. (125) have determined the partition coefficients of four organic acids between an aqueous phase and a preparation of basement membrane from rat kidney. The results were entirely consistent with the relative reabsorption rates of these compounds by nonionic diffusion.

Walser (126) has presented an extensive theoretical analysis of the kinetics of reabsorptive processes with emphasis on the influence of rate of tubular fluid flow on reabsorption. Of particular pertinence is the finding that a linear relationship between excretion and rate of urine flow is not neces-

sarily indicative of passive reabsorption. A substance reabsorbed by an active mechanism, which is at least half saturated, will give a similar pattern. Supplementary information is necessary to distinguish these mechanisms. In a second paper, Walser (127) considers the interrelationship of reabsorption of two solutes. He points out that this type of analysis is not always useful in distinguishing between various modes of reabsorption. However, it does seem quite likely that reabsorption of two solutes by independent active mechanisms that are partially saturated will result in a unique relationship of their excretory patterns.

Recent studies with specific compounds.—McIsaac (128) has found a remarkable species difference in the ability of renal slices to accumulate hexamethonium. McIsaac supports the earlier conclusion of Volle et al. (129) that uptake of hexamethonium is by the organic base transport system, but once in the tubular cells of some species the compound is largely bound at nonspecific sites. A somewhat similar phenomenon was encountered by Duggan (130) in a study of four thiazide diuretics. Cyclopenthiazide, was most markedly accumulated. This accumulation was as sensitive to inhibitors as the accumulation of chlorothiazide, hydrochlorothiazide, and trichlormethazide. In contrast to the other three compounds, cyclopenthiazide did not run out readily when the tissue was transferred to fresh medium or treated with inhibitors. Only cyclopenthiazide was concentrated in subcellular particles as compared to cytoplasm.

Dihydromorphine is secreted by the proximal tubules of dogs and monkeys by a mepiperphenidol-sensitive (organic base) mechanism (131). The excretion of a metabolite, probably a glucuronide, was quite sensitive to inhibition by probenecid. Radioactivity from C^{14}-neostigmine (132) and C^{14}-pyridostigmine (133) is rapidly excreted by rats, suggesting tubular secretion. Tubular secretion and inhibition by cyanine 863 with both compounds was proved in chickens. The excretion of dexamphetamine is dependent on the acidity of the urine (134). Active proximal secretion was not explored. The alterations in excretion rate by urinary pH changes influence the pharmacological responses to the drug (135).

The excretion of choline has been re-examined by Solomon (136) in an attempt to explain the urinary alkalinization induced by this substance. Secretion, when it occurred, seemed limited to the proximal tubule. Occasionally distal reabsorption could be demonstrated. It was suggested that choline secretion in the proximal tubule represents a choline for sodium exchange which competes with the normal hydrogen for sodium exchange.

Radioactivity from C^{14}-ethacrynic acid is secreted by proximal tubular cells (137). Excretion rate depends on urinary pH. Demethylchlortetracycline is secreted by a probenecid-sensitive mechanism (138). Spironolactone and its metabolite (dethioacetylated derivative) are secreted in the proximal tubule and reabsorbed distally (139, 140). Secretion was inhibited by bromcresol green. The uncorrected clearance of endogenous estriol (or its conju-

gates) exceeds filtration rate (141). Although the uncorrected clearances of estrone and estradiol (or their conjugates) are lower than GFR, these clearances like that of estriol can be depressed by probenecid.

The semi-synthetic antibiotic, cephaloridine, contains both a carboxyl group and a quaternary nitrogen. Clearances in various mammals average 0.6 or 0.7 times the filtration rate and are not very pH-dependent (142). However, individual values range from 0.3 to 1.2, suggesting bidirectional transport. Probenecid sensitive secretion is readily demonstrable in chickens. It is interesting to note that probenecid protects chickens and two species of mammals from the characteristic proximal tubular necrosis caused by cephaloridine.

Inhibition of renal carbonic anhydrase without the complication of enzyme inhibition in erythrocytes can be accomplished with proper doses of 2-benzenesulfonamido-1,3,4-thiadiazole-5-sulfonamide (143, 144). This is the result of efficient renal secretion which allows selective concentration in that organ, and extensive protein binding which limits diffusion into erythrocytes.

Walser & Rahill have demonstrated the interrelation of iodide (145) and bromide (146) excretion with that of chloride. It seems likely that all three ions are largely reabsorbed by a common mechanism.

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