RENAL EXCRETION OF DRUGS: TUBULAR TRANSPORT AND METABOLISM

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INTRODUCTION

The regulatory control of renal excretion of drugs and autopharmacological agents results from either renal tubular reabsorption of filtered solutes or the excretion of solutes into the lumen from peritubular blood. These tubular processes may be active transport processes or passive nonionic diffusion of lipid soluble substrates. Many drugs and autopharmacological agents such as biogenic amines are ionized at body pH and are relatively nonpermeant, but they are moved into and out of cells in directions opposite to their electrochemical gradients. These active transport processes across the renal tubule membrane have been demonstrated for organic cations such as tetraethylammonium (TEA) and catecholamines and for organic anions such as *p*-aminohippurate (PAH) and uric acid. The organic anion and cation transport systems are separate mechanisms which do not demonstrate mutual inhibition. The predominant transport of most organic ions in the renal tubule is in the secretory direction, which is in a direction opposite to the simultaneous movement of the inorganic cation, sodium. Specific examples of the selective tubular control of the excretion of certain drugs and autopharmacological agents will be described in this review. Increasing evidence is appearing that for some substances active transport can occur in the directions of both tubular excretion and tubular reabsorption; the amount appearing in the urine is the net result of these two opposing processes. Evidence for some bidirectional transports will be detailed in this review.

Intrarenal metabolism may modify the transport of drugs and autopharmacological agents. A drug may be transported into the renal tubular cell from the blood in one ionized form, be metabolized in the cell into a more polar compound of the same charge, to a neutral molecule, or to an oppositely charged compound. The metabolite will leave the cell by active transport or down a concentration gradient if it is diffusible.

Active membrane transports of organic ions across the renal tubule may

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be prototypes for similar transports in other cells such as those of the choroid plexus, brain, gut, eye. In nonrenal tissues it is difficult to identify an uphill active mechanism of transport and to assess the amount of nonspecific binding of substrates to the tissue, whereas in the renal tubule in vivo it is a simple matter to identify the rate of transcellular transport and to measure the concentrations in the peritubular blood pool and the luminal pool.

Several excellent reviews of the renal tubular transport of organic ions have appeared in the last decade (1–12).

Methods to Demonstrate Active Renal Tubular Transport

Reabsorptive transport.—The filtered organic ion may be either excreted completely into the urine or reabsorbed by the tubule by passive non-ionic diffusion or by active reabsorptive mechanisms, or both. Renal clearance and stop-flow procedures will detect a reabsorptive process. Passive mechanisms can be identified by an excretory response to alteration in urine volume or pH. Carrier mediated transports are identified by a response to competitive inhibitors. Cho & Cafruny (13) have introduced a technique to measure tubular reabsorptive patterns that depends on injection of the substrate into the ureter and forcing the bolus under pressure up into the tubular lumina. Following an interval, to allow reabsorptive processes to operate, the fluid is allowed to leave the tubular lumina and is fractionally collected from the ureter. Any selective loss of substrate from the injectate will indicate tubular reabsorption. In a similar way, the removal of substrates that have been inserted by micropuncture inoculation or perfusion may indicate reabsorptive transport.

Excretory transport.—Conventional renal clearance and stop-flow determinations will give evidence for tubular excretion in addition to glomerular filtration. Another technique to measure tubular secretion (excretion) makes use of the renal portal circulation in birds. This technique was developed by Sperber (14–17). Experimental substrates to be studied for active tubular excretion can be administered directly into a saphenous vein in the leg of the chicken and the injectate will go to the ipsilateral renal portal circulation and bathe the ipsilateral renal tubules. Urine is collected separately from each kidney. A substance that is secreted by the renal tubules will appear in the urine from the ipsilateral kidney in excess of that appearing in the urine from the contralateral kidney. This excess is divided by the amount infused. This value, described by Sperber as the Apparent Tubular Excretion Fraction, represents the rate of active tubular excretion. The technique has the advantage that it is not necessary to determine protein binding and filtration rate. Moreover, extremely small amounts of very potent physiologically active substances, autopharmacologic substances, or drugs can be studied by this approach since the substrate is infused into a

sequestered pool where the material moves from the saphenous vein directly to the renal parenchyma and is excreted, thus little enters the central circulation.

Renal metabolism.—The Sperber technique is useful to study renal metabolism in vivo. The renal metabolites of the infused substance appear in excess in the urine from the infused kidney. Consideration of extrarenal metabolites is eliminated. The technique can be used to measure in vivo the metabolic capacity of the renal parenchyma and the effects of drugs and chemicals on the function of certain renal enzymes.

Proximal Tubular Excretion and Metabolism of Anions

The organic anion transport mechanism has been exemplified by the well studied, synthetic substrate PAH. The structural requirements for a transportable anion have not yet been resolved. Anionic drugs are excreted by this transport system and their polar metabolites, particularly glucuronides and sulfates are transported by this mechanism. Probenecid is the selective competitor, which has a time honored role in the detection of active transport of anions.

The existence of a maximal tubular transport rate (Tm) for PAH has been attributed to saturation of a limited number of transport sites in the renal tubule. The concept of Tm_{PAH} was challenged by Deetjen & Sonnenberg (18). In a microperfusion study on proximal tubules in rat kidney they found a direct relation of PAH secretion and the volume of tubule perfusate. They also found no relation between the amount of PAH secreted and the length of the perfused segment. They proposed that Tm_{PAH} is limited by a maximal intraluminal PAH concentration but not by a limited number of carriers.

Tanner & Isenberg (19) studied PAH excretion in rat kidney by clearance, micropuncture, and stop-flow. They found that in micropuncture experiments the amount of secreted PAH increased progressively along the proximal pars convoluta. They reached intraluminal concentrations several times higher than those found by Deetien, and tubule volume flow rate in their experiments had little effect on the PAH secretion. They concluded that the tubular maximum for PAH is due to saturation of a limited number of proximal tubule secretory sites. In attempting to explain the difference between their findings and those of Deetjen, they suggested that the perfusion medium used by Deetjen may have limited PAH secretion, since it was lacking in K^+ and Ca^{++}.

Tune, Burg & Patlak (20) using perfused segments of rabbit nephrons in vitro found no relation between the volume of perfusate and PAH transport.

Park, Yoo & Hong (21) reported the ability of several organic acids to inhibit PAH and phenolsulfonphthalein (PSP) competitively in the rabbit

kidney slice. Their results confirm the generally accepted idea (4, 22) that effective inhibitors have higher affinity for the carrier and easily saturate it, but are only slowly transported themselves.

Chung, Park & Hong (23) used the rabbit kidney slice to study the effects of varying the cation concentration in the medium on the transport of PSP and PAH. The results showed a dependence of the slice uptake on the presence of adequate Na^+ and Ca^{++}. However, the removal of potassium was without effect.

Knoefel & Huang (24) have demonstrated synthesis of *m*-aminohippuric acid (MAH) from *m*-aminobenzoic acid (MAB) in the renal cortex of dogs. In stop-flow experiments the site of synthesis was coextensive with the proximal site of transport. The synthetic mechanism did not affect the synchronous transport of PAH. Diodrast inhibited the excretion of MAH formed from exogenous MAB and this was taken as evidence for a sensitive transport site on the luminal border.

Catechol (dihydroxybenzene) was actively transported by the renal tubule in the chicken (25). This transport could not be inhibited by organic acid or organic base competitors. Catechol is a weak acid and un-ionized at body pH and hence should not fall into the category of organic anion or organic cation transports. It was found that all of the infused catechol appeared as ionized renal metabolites in the urine, about one-half as glucuronide and one-half as ethereal sulfate. When these glucuronide and sulfate fractions were reinjected into the renal portal circulation in chickens, it was found that they were transported from blood to urine by an active transport and that this transport could be inhibited by probenecid. Thus the transcellular transport of catechol glucuronide and ethereal sulfate was inhibited by probenecid, but the transport of the intrarenally formed conjugates was not inhibited. The two sides of the renal tubule cell have long been a focus of discussion in the attempts to localize the transport site for anions. These two distinct sites have been referred to as peritubular and luminal. In these catechol experiments the sites could also be referred to as pre-metabolic and post-metabolic. These results with catechol support a view that the transport site is on the peritubular (pre-metabolic) side only. Stop-flow analysis of the site of excretion of catechol revealed that the catechol conjugates appeared at a site distal to the site of excretion of simultaneously administered PAH or norepinephrine (26).

Riboflavin excretion in the dog occurred by renal proximal tubular excretion by a probenecid sensitive system as well as by glomerular filtration (27). Some renal storage of riboflavin 5′ phosphate was detected following infusion of exogenous riboflavin.

It seems reasonable to suspect that the organic anion transport mechanism in the renal tubule functions physiologically to transport endogenous anion substrates. Physiologic roles are suggested by the following studies. Barac-Nieto & Cohen (28) studied the renal extraction from the blood of nonesterified fatty acid (NEFA) in vivo in dogs and measured the effect of

added probenecid or chlorothiazide. Both drugs decreased renal NEFA uptake. Only chlorothiazide prevented sodium reabsorption. Balagura & Stone (*29*) have studied renal extraction and renal tubular transport of alpha-ketoglutarate in dog kidneys in vivo and found net reabsorption and net renal extraction of alpha ketoglutarate in normal acid base balance, which was converted to net secretion by the simultaneous administration of citrate or during acute alkalosis. Pakarinen & Runeberg (30) studied the uptake and metabolism of alpha ketoglutarate and citrate in guinea pig kidney cortical slices. They found that PSP and probenecid affected the transport and metabolism of citrate and alpha ketoglutarate. Orringer, Weiss & Preuss (31) reported that azotemic sera from nephrectomized rats depressed the uptake of Na-iodohippurate into rat kidney slices. They propose that endogenous organic anions in azotemic sera such as lactate are responsible for the competitive inhibition of hippurate transport. Weiner, Roth & Skulan (32) compared the effect of 2,4 dinitrophenol (DNP) and sodium cyanide on renal PAH secretion in the dog in vivo. DNP reduced T_{PAH}/GFR but cyanide did not. However, only cyanide reduced sodium reabsorption at the doses given. In gouty human subjects the renal clearance of oxipurinol, the chief metabolite of allopurinol in man and dog, was less than 20% of the GFR (33). Oxipurinol is not bound to plasma proteins. Probenecid increased the clearance of oxipurinol. In the mongrel dog the renal clearance of oxipurinol was about one-half the glomerular filtration rate. Xanthine uptake in rabbit renal cortex slices has characteristics resembling the organic anion transport system, however, uric acid did not inhibit xanthine uptake (34).

Bimodal transport.—Renal tubular secretion of creatinine has been demonstrated in several species. A unique feature of the transport has been that it is inhibited by both anionic and cationic competitors. O'Connell, Romeo & Mudge (35) found that creatinine secretion in the dog tubule was sensitive to both types of competitors. Similar results were obtained in the chicken (36) and the guinea pig (37). It was originally suggested that the zwitterion form was being transported. Other transportable ions that exist as zwitterions in the body are catecholamines, riboflavin, and thiamine. Although direct evidence is not yet available, simultaneous transport of the compound and an oppositely charged metabolite would seem a possibility to explain this "bimodal transport." The competitor inhibits the transport of its appropriate substrate.

Probenecid

Probenecid was developed for the purpose of specifically competing for the anion transport system of the renal tubule that excretes penicillin. Subsequently, the uricosuric effect of probenecid was recognized and probenecid has become a common therapeutic tool for the treatment of hyperuricemia and gout (38). Currently the metabolism and excretion of probenecid are being studied. Perel et al (39) found about 25% of orally administered

probenecid excreted as acyl glucuronide in man and only a small amount of probenecid was excreted unchanged. About 80% of the orally administered C14-probenecid could be accounted for in urine and about half of this was found to consist of metabolites more polar than the parent drug. The renal clearance of probenecid acyl glucuronide was shown to be about one-third that of creatinine clearance. An important question seems to be whether the acyl glucuronide or the other unidentified metabolites of probenecid have significant pharmacologic effects (40). The anionic metabolites of biogenic amines are transported across the choroid plexus by a probenecid sensitive system. One might invoke a unitarian hypothesis for the active transport across neuronal and other nonrenal biologic membranes of organic anions and cations which proposes a mechanism the same as the one so well displayed in the renal tubule epithelium. Future studies will indicate whether probenecid will provide clinical usefulness in modifying the distribution of the metabolites of biogenic amines in the nervous system.

Proximal Tubular Excretion and Metabolism of Cations

A number of drugs and autopharmacologic substances are excreted by the renal tubule in the mammal by the organic cation transport system. Some substrates whose transport by the cation transport system in the kidney has been demonstrated are the synthetic quaternary ammonium compound tetraethylammonium (TEA) and naturally occurring cations such as N-methylnicotinamide, choline, catecholamines, and thiamine. Selective competitors used to identify cation transport are cyanine-863, quinine, and mepiperphenidol.

Dopamine was excreted by the renal tubule by this cation transport in the dog when administered into the renal artery to minimize extrarenal metabolism (41). Norepinephrine, epinephrine, and dopamine were excreted by the proximal tubule in dog stop-flow experiments (42). In a study of structure/transport relationships of a number of analogs of ethylamine in the Sperber chicken preparation, it was evident that the efficiency of excretory transport was enhanced by the presence of the phenyl ring and further by the presence of ring hydroxyls (43). The transport of all of these amines was inhibited by cyanine-863, indicating a cation transport mechanism. Probenecid could also weakly inhibit the transport of these analogs, indicating a simultaneous anionic transport component. The most likely explanation of this dual transport mechanism for catecholamine is that there are two species being transported simultaneously, the original amine and its acid metabolites formed by the kidney. If it is indeed true that probenecid is inhibiting the exit of intrarenally formed anionic metabolites, this would imply that there is an active transport step for anions at the luminal border.

An experimental proof of this suggestion of a luminal postmetabolic transport step is available for one biogenic amine, phenylethylamine (PEA) using the chicken kidney, in vivo. The transport of the cation PEA was insensitive to quinine (44, 45). Analysis of the urine during PEA infusion

revealed that all of the PEA was metabolized intrarenally to anionic metabolites. The transport of PEA was very sensitive to probenecid inhibition, hence it can be concluded that the luminal transport of anionic metabolite, phenylacetic acid, is probenecid sensitive.

Cocaine, which blocks the reuptake of catecholamines into the adrenergic neuron and perhaps into the effector cell, was an effective blocker of the renal tubular transport of catecholamines and TEA, indicating for cocaine's effect a cationic transport mechanism. There was, however, one exception in that cocaine did not block the transport of PEA. Cocaine did not block the entry of PEA into the peritubular side of the cell, and since cocaine does block the transport of TEA, this localizes the cation transport to the luminal side of the cell.

Ross et al (46) compared the characteristics of uptake and run-out for anion and cation systems using PAH and NMN in dog renal slices. There was no evidence of countertransport. Using β-haloalkyamine (47) as a selective irreversible cation transport competitor, Ross et al (48) have made the first attempt to isolate the protein associated with the transport of organic cations in mammalian kidneys. Using osmotic shock, attempts were made to isolate and purify a protein fraction associated with a transportable cation (49). Using structural analogs to detect inhibition of NMN uptake Reynard (50) detected a relation of alkyl chain length and hydrophobic character of reversible and irreversible agents. McIsaac (51) found a large variation from species to species in the ability of renal slices to take up mono and bisquaternary ammonium ions.

The renal tubule can actively transport choline in the dog and chicken (45, 52, 53). Using the Sperber technique, no tubular excretion of choline occurred when the amount of choline being infused was less than 8.6×10^{-8} mol/min. At these low infusion rates the infused choline was converted to betaine by the renal cells. Betaine was not actively transported by the renal tubule. At infusion rates above 1×10^{-5} mol/min tubular excretion of choline was found. Tubular transport of choline was inhibited by cationic competitors.

Choline is a biologically useful cation with several physiologic roles. Conservation of choline would seem to be desirable. These results suggest that the kidney conserves this methyl donor molecule at physiologic levels in plasma by metabolizing the choline that enters the renal cell from the peritubular blood to the nontransportable metabolite, betaine. On the other hand, when the plasma level rises above physiological levels, the renal enzyme capacity for choline metabolism is overloaded and the nonmetabolized choline is actively transported across the renal tubule in the direction of excretion. The tubular excretion of choline assists in lowering the elevated body stores of choline. Thus, there seems to be a renal mechanism for homeostasis of plasma choline.

The renal tubular excretion and simultaneous metabolism of morphine represents one of the most thoroughly worked out examples of renal drug

excretion and metabolism. Way & Adler (54) concluded that morphine and its metabolites were excreted primarily by the kidney. Baker & Woods (55) suggested that morphine may be excreted by the tubule in the dog. Hug et al (56) demonstrated tubular excretion of dihydromorphine by a cation transport system in dog and monkey using clearance and stop-flow studies. In this study mepiperphenidol reduced secretion in vivo and accumulation by slices in vitro of free morphine. Both mepiperphenidol and probenecid decreased the secretion in vivo of conjugated morphine, which was presumed to contain an acidic moiety (glucuronic acid) as well as basic nitrogen group. May, Fujimoto & Inturrisi (57) studied the renal excretion of morphine using the Sperber chicken technique. They found that morphine was excreted by the renal tubule by a cationic transport mechanism and a renal metabolite of morphine was formed. Watrous, May & Fujimoto (58) identified the metabolite as morphine ethereal sulfate. Probenecid did not inhibit the transport of morphine or the metabolite being formed intrarenally. However, when morphine ethereal sulfate was infused into the leg vein, its transcellular transport was inhibited by probenecid. They concluded that probenecid must be acting only on the peritubular membrane transport of morphine ethereal sulfate. The transcellular transport of morphine was inhibited only by cationic competitors.

Using the Sperber technique, the effect of catechol on the simultaneous renal transport and metabolism of morphine was studied (59). Catechol is completely metabolized by the renal cell to glucuronides and sulfates. Catechol eliminated the renal formation of morphine ethereal sulfate while the excretion of unmetabolized morphine was increased. The net transport of the carbon label from the morphine was unchanged by the introduction of catechol. When morphine ethereal sulfate was infused into the renal portal circulation, catechol did not affect its transport. These results support the idea of substrate competition by catechol at the metabolic site for sulfation.

Hakim & Fujimoto (60) studied the effects of SKF 525A, an inhibitor of enzyme activity, on the renal tubular transport of morphine and on the production of the metabolite, morphine ethereal sulfate using the chicken kidney, in vivo. It was found that SKF 525A produced a large fall in the transport of the carbon label during the infusion of labeled morphine, and the excretion of both morphine and morphine ethereal sulfate was reduced. SKF 525A did not affect the renal tubular transport of infused morphine ethereal sulfate. Thus, SKF 525A did not inhibit metabolism of morphine in the kidney, for that would have resulted in a reduction in morphine ethereal sulfate produced and an increase in the free morphine excreted. It was concluded that SKF 525A prevented access of morphine to the cell by preventing its membrane transport presumably at the peritubular border. SKF 525A inhibited the renal tubular transport of TEA, further supporting its role as an inhibitor of the cation transport mechanism. These results suggest a peritubular (premetabolic) site for morphine transport.

Marchand, Cantin & Côté (61) reported that when morphine sulfate was

administered chronically to rats, their renal slices failed to accumulate PAH. This result suggests the accumulation of a blocking load of the anionic metabolites of morphine. When they looked at the gross and microscopic picture of these kidneys they found massive lesions in the renal cortex, medulla, and papilla.

Sanner & Wortman (62) demonstrated the renal tubular excretion of 5-hydroxytryptamine (5HT) by the chicken kidney in vivo. This transport of 5-HT was found by Hakim, Watrous & Fujimoto (63) to be inhibited by mepiperphenidol and quinine, suggesting an organic cation transport mechanism. The infused 5HT was metabolized in the renal tubule to 5-hydroxyindolacetic acid (5HIAA). When 5-HIAA was infused into the renal portal circulation it was actively secreted by the organic anion transport system, as evidenced by a block of this transport by probenecid. However, probenecid did not affect the excretion of 5-HIAA formed intrarenally from 5-HT. They concluded that the transport site of 5-HIAA and the site for its block by probenecid is on the peritubular (pre-metabolic) border of the cell.

Attempts to localize the active transport step for organic anions and cations by the interpretation of results from such studies as are reported here, reveal that both the peritubular (pre-metabolic) step and the luminal (post-metabolic) step may be invoked depending on the substrates and the competitor used. The event of intrarenal metabolism in these studies may give an additional dimension to aid in transport localization. The inability of probenecid to inhibit the transport of anionic metabolites formed intrarenally from catechol, morphine, and 5-HT provides a model that suggests only a peritubular (pre-metabolic) transport step for anions. However, probenecid inhibition of the transport of phenylacetic acid formed intrarenally from PEA suggests a luminal (post-metabolic) site for anion transport. The Tm may have a higher capacity at the luminal side. Perhaps there is active transport for anions at both sites, but probenecid is incapable of competing with the high Tm at the luminal side when the substrate is provided only intrarenally. An attractive argument to support the idea that there is an active transport site for anions on the luminal (post-metabolic) side of the cell is based on the well recognized fact that conjugates such as glucuronides have been associated with accelerated excretion from the body. Teleologically it would serve no useful purpose to produce a conjugate within the cell and then to have a barrier on the luminal border to reduce its exit.

The localization of cation transport to the luminal (post-metabolic) side of the cell was suggested by the lack of effect of quinine and cocaine on PEA entry into the metabolic site of the renal cell, before it is metabolized to phenylacetic acid. However, it was possible to block the entry of morphine into the cell, suggesting a peritubular (pre-metabolic) cation transport step.

Bidirectional Tubular Transport

Increasingly, evidence is appearing to indicate simultaneous active bidirectional transport for a transportable organic ion. The evidence is not easy

to obtain in a quantitative fashion, particularly in relation to the physiological state. The stop-flow patterns under appropriate loading can provide qualitative evidence for bidirectional movement. Competitive inhibitors may unmask net movement in one direction. Simultaneous bidirectional active transport of a single substrate by a single cell or by a single segment of the kidney has been suggested by Berglund (64); Kinter (65); Gutman & Yu (66); and Beechwood, Berndt & Mudge (67). These earliest studies were reviewed by Weiner (9, 11, 12). Since that review more evidence for simultaneous bidirectional active transport has accumulated.

Zins & Weiner have demonstrated bidirectional active transport of taurocholate by the proximal tubule of the dog (68). In earlier work (69) it had been shown that the renal excretion of bile salts followed the pattern of glomerular filtration and tubular reabsorption by active transport in the proximal tubule. Bile salts demonstrated competitive inhibition of the organic anion secretory system. Subsequent studies clearly demonstrated that the bile salt, taurocholate, can itself be transported actively from the peritubular blood into the urine. The evidence was obtained by injection of taurocholate into the renal artery during ureteral occlusion in a stop-flow experiment in dogs. Thus net tubular excretion of taurocholate was apparent when taurocholate concentration was elevated in peritubular blood and when tubular reabsorption was minimized. This secretory flux of taurocholate was blocked by PAH and converted to net reabsorption. This suggested that the secretory flux was mediated by the organic anion secretory mechanism.

Another model for demonstrating bidirectional transport in the proximal tubule of the dog is the behavior of *m*-hydroxybenzoate (*m*-HBA). May & Weiner (70) found that in dogs the clearance of *m*-HBA corrected for protein binding can equal glomerular filtration rate when plasma concentrations are low. At higher concentrations net tubular reabsorption occurs. In spite of net reabsorption, administration of PAH or probenecid reduces clearance further. In stop-flow experiments with low plasma concentration, net proximal secretion is seen even if free flow clearances are less than GFR. This secretion is inhibited by PAH and probenecid. In stop-flow experiments with high plasma concentration, net reabsorption is seen. This is augmented by inhibition of secretion which also allows localization of the process to the proximal tubule. Therefore, *m*-HBA is actively transported both by the proximal tubular organic anion secretory and reabsorptive mechanisms. In the cebus monkey (71) bidirectional active transport of *m*-HBA was demonstrated. The compound is a powerful inhibitor of the excretion of uric acid.

Excretion of uric acid has components of glomerular filtration, tubular reabsorption, and tubular excretion as revealed in different species by studies with competitive inhibitors. Zins & Weiner (72) clearly demonstrated bidirectional urate transport by active processes in the proximal tubule in the mongrel dog. During stop-flow, intra-arterial injection of uric acid re-

sulted in urate secretion which could be prevented by PAH, chlorothiazide, and salicylate. From this it was concluded that there is a secretory flux of uric acid which is mediated by the organic anion transport system. There was no evidence of distal secretion of uric acid. Kramp et al (73) studied the intrarenal transport of urate-2-C^{14} when microinjected into rat nephrons. Proximal tubular reabsorption of urate was evident. Probenecid, pyrazinoate, and PAH increased urine recovery of urate-2-C^{14} injected into the proximal tubule. The reabsorptive mechanism demonstrated saturation kinetics. No evidence for urate secretion was found.

Roch-Ramel & Boudry (74) microperfused C^{14}-urate into the lumen of nephrons. Probenecid sensitive reabsorption was demonstrated. Tubular secretion of urate was indicated by the appearance of C^{14}-urate in the lumina of proximal tubules when infused into the aorta. PAH depressed the entry of C^{14}-urate. Mudge et al (75) using renal clearance and stop-flow analysis in mongrel dogs concluded that uric acid had a bidirectional active transport at a proximal site. There was no evidence for distal secretion. Inulin was used as a reference to avoid any interference by creatinine, which itself is transportable. Pyrazinoic acid had no demonstrable action on distal tubular function, but lowered urate concentration in stop-flow samples of proximal origin.

Berger & Yu (76) studied uric acid excretion using the Chinard technique of intra-renal artery injection in mongrel and Dalmatian dogs. The evidence obtained suggested the presence of renal tubular reabsorption and secretion for uric acid in mongrel dogs and shows evidence for secretion in the Dalmatian. Creatinine was used as a marker substance.

Fanelli et al (77) showed that the renal clearance of urate in the chimpanzee is similar to that in man. Probenecid partially inhibits tubular reabsorption of urate. Pyrazinoic acid causes marked urate retention. Interaction of pyrazinoic acid and probenecid led to an interpretation that suggests cellular biosynthesis of urate in the renal tubule. There is bidirectional transport of uric acid in the guinea pig (78).

Maroske & Weiner (79) determined that the low renal excretion of zoxazolamine, a powerful uricosuric agent in man, is predominantly the result of extraordinarily rapid passive, back diffusion of the filtered drug when studied in the dog.

Although net tubular secretion of PAH is normally found, it is often slightly less than 100%. Perhaps some tubular reabsorption of the filtered and secreted PAH could occur simultaneously. Cho & Cafruny (13) interpret their experimental results in the dog using their technique of retrograde intraluminal injection to indicate renal tubular reabsorption of PAH in the proximal tubule by a carrier mediated process that was inhibited by probenecid.

Baines et al (80) investigated bidirectional movement of PAH in vivo in rats. When radioactive PAH was microinjected into superficial proximal convolutions, its recovery from the urine was complete in 8 to 20 minutes.

However, when the animal was preloaded with nonradioactive PAH, 10% of the proximally microinjected PAH was not recovered. It was concluded that there was a transtubular efflux of PAH from the lumen and that PAH preloading competitively inhibited its influx back into the tubular urine.

Tanner (81) localized the sites of tubular transport of PAH and Diodrast (iodopyracet) in the Necturus kidney perfused with Ringer solution. Net PAH reabsorption was found in the proximal tubule. Kidneys of intact Necturi reabsorbed both PAH and Diodrast. Net Diodrast secretion by the proximal tubule was induced by high plasma PAH concentrations. Diodrast injected into the tubule was completely reabsorbed and this reabsorption was inhibited by PAH.

Huang & Woosley (82) found L-glucose to be secreted by the proximal tubule of the dog and rat in steady state clearance experiments and in dog stop-flow experiments. Probenecid did not inhibit this tubular transport, but phlorizin did. Elevation of D-glucose levels in the blood increased the L-glucose tubular excretion. It was postulated that active reabsorption of D-glucose and active secretion of L-glucose occur in the proximal tubule cell by the same system. Free-flow micro-puncture experiments and microperfusion by Baumann & Huang (83) support the finding of proximal tubular secretion of L-glucose inhibited by phlorizin.

Williams & Huang (84) studied the tubular transport of L- and D-forms of the amino acid tryptophan. L-forms of amino acids are known to be reabsorbed by the renal tubule when studied by clearance methods. Clearance experiments in dogs revealed net reabsorption of L-tryptophan and net secretion of D-tryptophan and N-acetyl-L-tryptophan. The net reabsorption of L-tryptophan is probenecid sensitive. Stop-flow experiments revealed that all three isomers showed secretory peaks in the proximal tubule and these peaks could be prevented by the presence of probenecid. It was concluded that a bidirectional movement exists for L-tryptophan in the proximal tubule, but the two limbs of transport are not similar in mechanism since probenecid blocks only secretion.

Development of Transport in the Newborn: Induction of Transport

Drug excretion in the newborn is known to be somewhat different from that in the adult. Drug metabolism also varies with age. The post-natal development of renal function in the dog and rat have been elegantly studied in vivo (85, 86). Filtration rate and PAH transport have been measured. PAH and TEA renal slice uptake was studied in chick embryo mesonephros and metanephros and in renal tissue after hatch (87). Mesonephros accumulation was high in the embryo. The metanephros accumulation was low in the embryo and increased progressively to adult levels.

The anion accumulation mechanism can be enhanced or induced during the newborn period by previous loading with organic anions (88–91). This interesting effect has been demonstrated in several species by Hirsch &

Hook in slice uptake studies. Pretreatment with either penicillin or PAH during the sensitive period resulted in an amplification of slice uptake of PAH. The increased PAH uptake following loading was prevented by treatment with cycloheximide. It was not possible to enhance the cation transport mechanism.

Cholecystographic Agents

Cholecystographic agents are actively transported in the liver and kidney. There is occasional serious nephrotoxicity associated with their use, which may bear a relation to the renal transport and metabolism of these agents. There is a pronounced uricosuric action of the oral graphic agents in humans; the role this could play in nephrotoxicity in those who are dehydrated during the diagnostic test is discussed (92, 93).

Mudge, Berndt & Wade (94) studied several radiopaque oral cholecystographic agents. All of the agents used inhibited PAH uptake in rabbit kidney slices. In dog renal clearance studies, some agents did not result in much inhibition of PAH transport.

Berndt & Mudge (95) studied the renal excretion of iodipamide, an agent used for intravenous cholangiography in humans. In the rabbit, iodipamide gave evidence of renal tubular excretion that was probenecid sensitive, suggesting the organic anion transport mechanism. In the dog neither classical clearances, nor stop-flow analysis revealed any tubular excretion or reabsorption. This is a unique species differential for organic anion transport in mammals. The nature of the renal excretion mechanisms in man have not been defined.

The metabolism and biliary and renal excretion of iophenoxic acid (Teridax) in the dog was studied, although it is no longer used clinically since it was found to have a plasma half-life of 2½ years (96, 97). It differs from its clinically useful analog, iopanoic acid (Telepaque) only by a 3-OH instead of a 3-NH_2 group. The major excretory products of iophenoxic acid in bile and urine were found to be glucuronide conjugates.

Miscellaneous

Clarkson & Magos (98) have studied the mechanism by which renal tissue selectively accumulates some 70% of injected mercuric salts. They used rats and studied kidney function in vivo. They sought to differentiate between two mechanisms; (*a*) a tissue component with some uniquely high affinity for mercury or (*b*) an active accumulation requiring metabolic energy. Only DNP inhibited mercury uptake. Since sodium fluoroacetate, probenecid, sodium fluoride, and sodium malonate had no effect, it was concluded that the mercury uptake mechanism is related to energy dependent processes. DNP did not enhance the excretion of mercury.

Dybing & Kolberg (99) reported that 2,4-D (Dichlorophenoxyacetate) could decrease the Tm_{PAH} in rabbits. The Tm_{PAH}/C-Cr fell from 0.91 to 0.44. Pritchard & Kinter (100) studied the mechanism of excretion of DDT

and the major polar metabolite of DDT, namely DDA [bis (p-chlorophenyl) acetic acid]. Using the isolated renal tubule preparation in flounder kidney tubules, they showed that DDA inhibited the renal tubular transport of the organic acid chlorophenol red (CPR). Thus, DDA is presumably excreted by the organic anion transport mechanism. DDT, which lacks a carboxyl group, was not able to inhibit the organic anion transport of CPR. Roan et al (101) followed the renal excretion of orally administered DDT and metabolites of DDT in humans. Wolfe et al (102) described the urinary excretion of insecticide metabolites in spraymen over a three year period in occupational exposure situations with DDT alone or DDT and parathion.

Smalley & Radeleff (103) described an interesting renal drug interaction in sheep and swine. Representative compounds from the major classes of organic insecticides were given to sheep and swine to establish minimal toxic doses. Addition of diazoxide reduced the minimal toxic dose of the three insecticides in swine, from 20-fold to 3-fold. Diazoxide did not affect the minimal toxic dose of the insecticides in sheep. Diazoxide, a nondiuretic thiazide analog, has been suggested as an agent to reduce the loss of body fluids in livestock during the stress of shipping, marketing, and slaughter, since it produces fluid retention. Presumably, diazoxide prevented the renal excretion of the insecticides in swine.

Aronson & Ahrens (104) have studied the renal excretion of ethylenediaminetetraacetate in conscious dogs, calves, and rats. The data indicated that the mechanism for the renal excretion of EDTA is glomerular filtration. The clearance ratio of EDTA/inulin was 0.94–0.97. The clearance ratio was unaffected by urine pH, rate of urine flow, or by added PAH.

Eide (105) performed stop-flow experiments with Cr^{51}-EDTA in dogs and could find no evidence for tubular reabsorption of Cr^{51}-EDTA. He concluded that the renal clearance of Cr^{51}-EDTA was 10% lower than inulin because the isotope EDTA complex decomposed during storage.

LITERATURE CITED

1. Sperber, I. 1959. *Pharmacol. Rev.* 11:109–34
2. Peters, L. 1960. *Pharmacol. Rev.* 12:1–35
3. Forster, R. P. 1961. *Kidney Cells.* In: *The Cell,* vol. 5, pt. 2. ed. J. Brachet, A. E. Mirsky. New York: Academic
4. Wilbrandt, W., Rosenberg, T. 1961. *Pharmacol. Rev.* 13:109–83
5. Weiner, I. M., Mudge, G. H. 1964. *Am. J. Med.* 36:743–62
6. Despopoulos, A. 1965. *J. Theoret. Biol.* 8:163–92
7. Forster, R. P. 1965. *Ann. Rev. Physiol.* 27:183–232
8. Baer, J. E., Beyer, K. H. 1966. *Ann. Rev. Pharmacol.* 6:261–92
9. Weiner, I. M. 1967. *Ann. Rev. Pharmacol.* 7:39–56
10. Forster, R. P. 1967. *Fed. Proc.* 26: 1008–19
11. Weiner, I. M. 1971. *Handbook of Physiology, Renal Physiology.* ed. R. W. Berliner, J. Orloff, In press. Washington, D.C.: Am. Physiol. Soc.
12. Weiner, I. M. 1971. *Handbook Exper. Pharmacol.* ed. B. B. Brodie, J. Gillette, 28/1:328–53
13. Cho, K. C., Cafruny, E. J. 1970. *J. Pharmacol. Exp. Ther.* 173:1–12
14. Sperber, I. 1946. *Nature* 158:131
15. Sperber, I. 1948. *Lantbruks-Hoegsk. Ann.* 15:317–49
16. Lindahl, K. M., Sperber, I. 1956. *Acta Physiol. Scand.* 36:13–16
17. Sperber, I. 1960. *Biology and Com-*

parative Physiology of Birds. ed. A. J. Marshall. Vol. I. Chap. XII Excretion. New York: Academic
18. Deetjen, P., Sonnenberg, H. 1965. *Pfluegers Arch. Ges. Physiol.* 285:35–44
19. Tanner, G. A., Isenberg, M. T. 1970. *Am. J. Physiol.* 219:889–92
20. Tune, B. M., Burg, M. B., Patlak, C. S. 1969. *Am. J. Physiol.* 217: 1057–63
21. Park, Y. S., Yoo, H. S., Hong, S. K. 1971. *Am. J. Physiol.* 220:95–99
22. Sperber, I. 1954. *Arch. int. Pharmacodyn. Ther.* 97:221–31
23. Chung, S. T., Park, Y. S., Hong, S. K. 1970. *Am. J. Physiol.* 219:30–33
24. Knoefel, P. K., Huang, K. C. 1969. *Proc. Soc. Exp. Biol. Med.* 130: 914–19
25. Quebbemann, A. J., Rennick, B. R. 1968. *Am. J. Physiol.* 214:1201–04
26. Rennick, B. R., Quebbemann, A. J. 1970. *Am. J. Physiol.* 218:1307–12
27. Jusko, W. J., Rennick, B. R., Levy, G. 1970. *Am. J. Physiol.* 218: 1046–53
28. Barac-Nieto, M., Cohen, J. J. 1968. *Am. J. Physiol.* 215:98–107
29. Balagura, S., Stone, W. J. 1967. *Am. J. Physiol.* 212:1319–26
30. Pakarinen, A., Runeberg, L. 1969. *Biochem. Pharmacol.* 18:2439–52
31. Orringer, E. P., Weiss, F. R., Preuss, H. G. 1971. *Clin. Sci.* 40: 159–69
32. Weiner, I. M., Roth, L., Skulan, T. W. 1971. *Am. J. Physiol.* 221: 86–91
33. Elion, G. B., Yü, T. F., Gutman, A. B., Hitchings, G. H. 1968. *Am. J. Med.* 45:69–77
34. Berndt, W. O. 1970. *Nephron* 7: 339–49
35. O'Connell, J. M. B., Romeo, J. A., Mudge, G. H. 1962. *Am. J. Physiol.* 203:985–90
36. Rennick, B. R. 1967. *Am. J. Physiol.* 212:1131–34
37. Arendshorst, W. J., Selkurt, E. E. 1970. *Am. J. Physiol.* 218:1661–70
38. Gutman, A. B. 1966. *Advan. Pharmacol.* 4:91–142
39. Perel, J. M., Dayton, P. G., Yü, T. F., Gutman, A. B. 1971. *Eur. J. Clin. Pharmacol.* 3:106–12
40. Perel, J. M., Cunningham, R. F., Fales, H. M., Dayton, P. G. 1970. *Life Sci.* 9:1337–43
41. Rennick, B. R. 1968. *Am. J. Physiol.* 215:532–34
42. Rennick, B. R., Quebbemann, A. J. 1970. *Am. J. Physiol.* 218:1307–12
43. Quebbemann, A. J., Rennick, B. R. 1969. *J. Pharmacol. Exp. Ther.* 166:52–62
44. Quebbemann, A. J., Rennick, B. R. 1970. *J. Pharmacol. Exp. Ther.* 175:248–58
45. Rennick, B. R., Quebbemann, A. J. 1971. *Renal Pharmacology.* ed. J. W. Fisher, E. J. Cafruny, 67–84. Appleton-Century-Crofts: Meredith
46. Ross, C. R., Pessah, N. I., Farah, A. 1968. *J. Pharmacol. Exp. Ther.* 160:375–80
47. Ross, C. R., Pessah, N. I., Farah, A. 1968. *J. Pharmacol. Exp. Ther.* 160:381–86
48. Ross, C. R., Pessah, N. I., Farah, A. 1969. *J. Pharmacol. Exp. Ther.* 167:235–42
49. Magour, S., Farah, A., Sroka, A. 1969. *J. Pharmacol. Exp. Ther.* 167:243–52
50. Reynard, A. M. 1968, *J. Pharmacol. Exp. Ther.* 163:461–67
51. McIsaac, R. J. 1969. *J. Pharmacol. Exp. Ther.* 168:6–12
52. Rennick, B. R. 1958. *J. Pharmacol. Exp. Ther.* 122:449–56
53. Acara, M. A. 1971. *Renal Tubular Transport of Choline and Related Compounds; Modifications Caused by Intrarenal Metabolism.* Ph.D. thesis. S.U.N.Y., Buffalo. 142 pp.
54. Way, E. L., Adler, T. K. 1960. *Pharmacol. Rev.* 12:383–446
55. Baker, W. P., Woods, L. A. 1957. *J. Pharmacol. Exp. Ther.* 120:371–74
56. Hug, C. C., Mellett, L. B., Cafruny, E. J. 1965. *J. Pharmacol. Exp. Ther.* 150:259–69
57. May, D. G., Fujimoto, J. M., Inturrisi, C. E. 1967. *J. Pharmacol. Exp. Ther.* 157:626–35
58. Watrous, W. M., May, D. G., Fujimoto, J. M. 1970. *J. Pharmacol. Exp. Ther.* 172:224–29
59. Watrous, W. M., Fujimoto, J. M. 1971. *Biochem. Pharmacol.* 20: 1479–91, 1971
60. Hakim, R., Fujimoto, J. M. 1971. *Biochem. Pharmacol.* 20:2647–63
61. Marchand, C., Cantin, M., Côté, M. 1969. *Can. J. Physiol. Pharmacol.* 47:649–55

62. Sanner, E., Wortman, B. 1962. *Acta Physiol. Scand.* 55:319–24
63. Hakim, R., Watrous, W. M., Fujimoto, J. M. 1970. *J. Pharmacol. Exp. Ther.* 175:749–62
64. Berglund, F. 1961. *Acta Physiol. Scand.* 52:276–90
65. Kinter, W. B. 1959. *Am. J. Physiol.* 196:1141–49
66. Gutman, A. B., Yu, T. F. 1961. *Trans. Assoc. Am. Physicians.* 74:353–65
67. Beechwood, E. C., Berndt, W. O., Mudge, G. H. 1964. *Am. J. Physiol.* 207:1265–72
68. Zins, G. R., Weiner, I. M. 1968. *Am. J. Physiol.* 215:840–45
69. Weiner, I. M., Glasser, J. E., Lack, L. 1964. *Am. J. Physiol.* 207: 964–70
70. May, D. G., Weiner, I. M. 1970. *Am. J. Physiol.* 218:430–36
71. May, D. G., Weiner, I. M. 1971. *J. Pharmacol. Exp. Ther.* 176:407–17
72. Zins, G. R., Weiner, I. M. 1968. *Am. J. Physiol.* 215:411–22
73. Kramp, R. A., Lassiter, W. E., Gottschalk, C. W. 1971. *J. Clin. Invest.* 50:35–48
74. Roch-Ramel, F., Boudry, J. F. 1971. *Fed. Proc.* 30:338
75. Mudge, G. H., Cucchi, J., Platts, M., O'Connell, J. M. B., Berndt, W. O. 1968. *Am. J. Physiol.* 215: 404–10
76. Berger, L., Yü, T. F. 1971. *Mt. Sinai J. Med.* 37:351–58
77. Fanelli, G. M. Jr., Bohn, D. L., Reilly, S. S. 1971. *Am. J. Physiol.* 220:613–20
78. Mudge, G. H., McAlary, B., Berndt, W. O. 1968. *Am. J. Physiol.* 214: 875–79
79. Maroske, D., Weiner, I. M. 1968. *J. Pharmacol. Exp. Ther.* 159:409–15
80. Baines, A. D., Gottschalk, C. W., Lassiter, W. E. 1968. *Am. J. Physiol.* 214:703–09
81. Tanner, G. A. 1967. *Am. J. Physiol.* 212:1341–46
82. Huang, K. C., Woosley, R. L. 1968. *Am. J. Physiol.* 214:342–47
83. Baumann, K., Huang, K. C. 1969. *Pfluegers Arch. Ges. Physiol.* 305: 155–66
84. Williams, W. M., Huang, K. C. 1970. *Am. J. Physiol.* 219:1468–75
85. Horster, M., Lewy, J. E. 1970. *Am. J. Physiol.* 219:1061–65
86. Horster, M., Valtin, H. 1971. *J. Clin. Invest.* 50:779–95
87. Rennick, B. R. 1969. *Am. J. Physiol.* 217:247–50
88. Hirsch, G. H., Hook, J. B. 1969. *Science.* 165:909–10
89. Hirsch, G. H., Hook, J. B. 1970. *Can. J. Physiol. Pharmacol.* 48: 550–56
90. Hirsch, G. H., Hook, J. B. 1970. *J. Pharmacol. Exp. Ther.* 171:103–08
91. Hirsch, G. H., Hook, J. B. 1970. *J. Pharmacol. Exp. Ther.* 174:152–58
92. Mudge, G. H. 1971. *N. Engl. J. Med.* 284:929–33
93. Mudge, G. H. 1970. *Invest. Radiol.* 5:407–12
94. Mudge, G. H., Berndt, W. O., Wade, D. N. 1971. *Proc. Soc. Exp. Biol. Med.* 136:309–12
95. Berndt, W. O., Mudge, G. H. 1968. *Invest. Radiol.* 3:414–26
96. Mudge, G. H., Strewler, G. J., Desbiens, N., Berndt, W. O., Wade, D. N. 1971. *J. Pharmacol. Exp. Ther.* 178:159–72
97. Wade, D. N., Desbiens, N., Strewler, G. J. Jr., Berndt, W. O., Mudge, G. H. 1971. *J. Pharmacol. Exp. Ther.* 178:173–79
98. Clarkson, T. W., Magos, L. 1970. *Biochem. Pharmacol.* 19:3029–37
99. Dybing, F., Kolberg, A. 1967. *Acta Pharmacol. Toxicol.* 25: Suppl. 4: 51
100. Pritchard, J. B., Kinter, W. B. 1970. *Bull. Mount Desert Island Biol. Lab.* 10:67–68
101. Roan, C., Morgan, D., Paschal, E. H. 1971. *Arch. Environ. Health* 22: 309–15
102. Wolfe, H. R., Durham, W. F., Armstrong, J. F. 1970. *Arch. Environ. Health* 21:711–16
103. Smalley, H. E., Radeleff, R. D. 1971. *Am. J. Vet. Res.* 32:345–48
104. Aronson, A. L., Ahrens, F. A. 1971. *Toxicol. App. Pharmacol.* 18:1–9
105. Eide, I. 1970. *Scand. J. Clin. Lab. Invest.* 26:373–80