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Renal Organic Anion Transport System: Pharmacological, Physiological, and Biochemical Aspects

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I. Introduction

MUCH of our present knowledge about the secretory function of the kidney is based on studies of the mechanism by which foreign organic anions are excreted. The kidney secretes a wide variety of organic anions, e.g. p-aminohippurate (PAH), phenolsulfophthalein (PSP) dyes, urate, and many drugs. Similar transport of organic anions occurs in other tissues of epithelial origin, viz. liver (123, 125, 455, 527), choroid plexus (347), and anterior uvea (14, 15, 22). The tubular secretion of organic anions is sharply differentiated from organic cations which are also secreted by the kidney, but which do not interfere with the transport of organic anions (355, 380). The organic anions and cations also differ in their response (stimulation/inhibition) to metabolites and metabolic inhibitors (161, 163, 295, 381).

The homogeneity of the anion system is currently under debate, and it appears probable that several different subsystems exist for the transport of various anions (section III A). This raises the question on what basis should this transport system be distinguished from that of other organic anions such as acidic amino acids, citrate cycle intermediates, and lactate. Although these latter compounds are reabsorbed by the kidney tubules, some of them [e.g. glutamate (404) and lactate (328)] are also transported from the peritubular to intracellular fluid across the basolateral membrane. In this review we shall operationally adopt as definition of the transport system(s) under consideration that the uptake of substrates of the system should be inhibitable by probenecid, PAH, or another representative substrate of the system.

The purpose of the present review is to consider the mechanisms of organic anion transport and their consequences for the renal excretion of drugs and endogenous substrates. In part II we review in detail the renal handling of some selected organic anions: PAH, urate, PSP dyes, and pharmacologically active compounds. In part III we consider general properties of the organic anion transport system. Emphasis in the review is placed on sites and modes of transtubular transport, metabolic energization, cation dependence, multiplicity of organic anion transport systems, and substrate specificity. Since many of the compounds, in addition to undergoing tu-

bular secretion, are reabsorbed by the kidney the mechanisms that underlie tubular reabsorption are briefly considered.

A comprehensive review of the excretion of organic anions was previously given by Weiner (501). Irish and Grantham (239) have recently reviewed the excretion and the organic anion transport system emphasizing the medical aspects. In older reviews Despopoulos (122) has extensively discussed common structural features of secreted organic anions while Sperber (455) presented comparative aspects of the secretory systems in kidney and liver of various species.

A. Medical and Physiological Importance of the Renal Organic Anion System

Table 1 shows a list of compounds that are substrates of the organic anion secretory system. Briefly, the main criteria that have been used to select the substrates of the system are: 1) inhibition of renal excretion (in vivo) or of energy dependent uptake of the compound by renal cells (in vitro) by probenecid, PAH, or another representative substrate of the system; and 2) demonstration of net tubular secretion, wherever such evidence is available (in vivo experiments, in the absence of predominant reabsorption). These criteria are further discussed in section I C and in the legend of table 1. It is seen from the table that an unusually wide range of substances are transported, including hippurates, sulfated and glucuronidated organic anions (group I), benzoates, and other aromatic and heterocyclic carboxylates (groups II, III, and IV), sulfonamides, and heterocyclic compounds capable of proton dissociation (groups V and VI), aromatic sulfonates (group VII), aromatic amino acids and derivatives (group VII), and a miscellaneous group (IX). The pharmacologically active compounds include diuretics, drugs with an effect on urate excretion, x-ray contrast agents, sulfonamides, and antibiotics (penicillins and cephalosporins). The presence of tubular secretion often potentiates the pharmacological effect of drugs with an intratubular site of action (diuretics and uricosurics).

The primary function of the renal organic anion secretory system seems to be elimination of foreign compounds that only are catabolized to a limited extent and that in high dosage may be regarded as toxic to the body. Substrates of the organic anion system are often conjugated with glycine, glucuronic acid, or sulfate prior to excretion (section II J). Apart from drugs, substrates may arise from the ordinary diet, from the metabolism of intestinal bacteria, or from endogenous compounds. Examples are hippurate, phenolsulfate, and sulfated or glucuronidated degradation products of steroid hormones (group I compounds). In the uremic state there is a large increase in the level of sulfate and glycine conjugated compounds (56, 361). In agreement with the view that the compounds are excreted by the organic anion system, uremic serum has a distinct inhibitory effect on PAH transport (58, 228, 313, 344). In addition to conjugated compounds the system is involved in the excretion of the metabolic end products, urate (group VII) and 5-hydroxyindoleacetate (group III). It may be involved in the excretion and possibly the intrarenal function of prostaglandins (46, 238, 379).

The role of the system, if any, in the homeostasis of physiologically active compounds such as pantothenate, folate and aromatic amino acids (group VIII), and ascorbate (group IX) is unknown. It has been debated whether the system may serve a function in supplying renal cells with nutrients such as fatty acids and dicarboxylic acids (94). This view is mainly based on the observation that renal uptake of α-ketoglutarate and fatty acids is inhibited by probenecid (11, 413). However, a consideration of recent evidence leads to the conclusion that these endogenous metabolites probably cannot be regarded as true substrates (section III B). On the other hand, an excess of intermediary metabolites, resulting from organic acidurias, may lead to the formation of glycine conjugates which probably are secreted by the kidney (20, 192).

In some cases the organic anion secretory system seems to be involved in the development of nephrotoxic effects. For instance the nephrotoxic effect of the antibiotic, cephalosporidine, involves the proximal tubules and is correlated with the intracellular accumulation of the compound arising from transport (476, 480). The organic anion transport system also may be involved in experimental damage to the proximal tubule by administration of dichromate (64, 214).

X-ray contrast agents for delineation of kidney or liver have been selected on the basis of their respective affinity for transport by liver and renal cells (14). The use of ^{125}I-hippuran scintigraphy of the kidneys to estimate the functional viability of renal cells during anuria or oliguria (208) and for transplantation purposes (405, 456) rests on the assumption that intracellular accumulation of organic anions is highly dependent on an optimal functioning of the metabolic machinery of the renal cells. In agreement with this view renal PAH accumulation is more susceptible to a curtailment of energy-producing processes than is Na^+ transport (307).

B. Evaluation of Experimental Techniques

1. In Vitro Methods. In comparison to other fields of renal investigation a major part of our knowledge has come from the study of in vitro preparations. The scope of these techniques in the study of organic anion transport is considered below.

A. ACCUMULATION OF ORGANIC ANIONS BY RESPIRING KIDNEY SLICES. The particular advantage of the technique, as compared to in vivo experiments, is that uptake can be measured under well controlled medium conditions. The procedure is simple and readily permits testing of organic anion transport under a variety of conditions that are not otherwise possible. Secondary effects such as changes in blood flow, plasma protein binding, toxicity of the compound in whole animal experiments, and hormonal interference are obviated. Drawbacks of the tech-

TABLE 1

Renal tubular transport of organic anions.

Organic Anions	Chemical Names	Demonstration of		
		Active Secretion		Reabsorption
		In vivo	*In vitro*	
I. Conjugated compounds				
A. Glycine conjugates				
1. Hippurate	Benzylaminoacetate	271, 442, 452		
2. *o*-Methoxyhippurate	2-Methoxybenzylaminoacetate	271		
3. *o*-Nitrohippurate	2-Nitrobenzylaminoacetate	271		
4. *m*-Nitrohippurate	3-Nitrobenzylaminoacetate	271		269, 274
5. 2-Hydroxy-5-nitrohippurate	2-Hydroxy-5-nitrobenzylaminoacetate	274		274
6. 2-Hydroxy-3-nitrohippurate	2-Hydroxy-3-nitrobenzylaminoacetate	274		274
7. *o*-Aminohippurate	2-Aminobenzylaminoacetate	271	122, 272	
8. *m*-Aminohippurate	3-Aminobenzylaminoacetate	442	122, 272	269
9. *p*-Aminohippurate	4-Aminobenzylaminoacetate	27, 98, 116, 442	102, 174, 407, 425, 433‡, 478, 528	253, 257, 471¶
10. *o*-Acetylaminohippurate	2-Acetylaminobenzylaminoacetate	326	326	
11. *p*-Acetylaminohippurate	4-Acetylaminobenzylaminoacetate	204, 300, 442	204	
12. *p*-Acetamidohippurate	3-Acetylaminobenzylaminoacetate		122, 272	
13. *p*-Acetamidohippurate	4-Acetylaminobenzylaminoacetate	442	122, 272	
14. Salicylurate	2-Hydroxybenzylaminoacetate	274, 406, 442, 504	122	274, 504
15. *m*-Nitrosalicylurate	3-Nitro-2-hydroxylamioacetate	274		
16. 5-Nitrosalicylurate	5-Nitro-2-hydroxylaminoacetate	274		
17. *m*-Hydroxyhippurate	3-Hydroxybenzylaminoacetate	271, 442		
18. *p*-Hydroxyhippurate	4-Hydroxybenzylaminoacetate	274, 442		
19. *o*-Iodohippurate	2-Iodobenzylaminoacetate	144, 145		
20. *n*-Propionylglycine	*n*-Propionylaminoacetate		421	
21. *n*-Butyrylglycine	*n*-Butyrylaminoacetate		421	
22. *n*-Valerylglycine	*n*-Pentylaminoacetate		407, 421	
23. *n*-Caproylglycine	*n*-Hexanoylaminoacetate		407, 421	
24. Isocaproylglycine	Hexanoylaminoacetate		407, 421	
25. *n*-Oenantheoylglycine	*n*-Heptanoylaminoacetate		407, 421	
26. *n*-Capryloylglycine	*n*-Octanoylamioacetate		407, 421	
27. Cinnamoylglycine	3-Phenyl-2-propenoylaminoacetate	442		
28. Glycocholate	N-(3α,7α,12α-Trihydroxy-24-oxocholan-24-yl)aminoacetate	442	17, 491	491§, 505
B. Other amino acid conjugates				
1. *p*-Aminobenzoyl-L-α-alanine	4-Aminobenzoyl-L-α-aminoproprionate	122	122	
2. *p*-Aminobenzoyl-L-α-alanine	4-Aminobenzoyl-D-α-aminopropionate	271	122	
3. *p*-Aminobenzoyl-β-alanine	4-Aminobenzoyl-β-aminopropionate	271		
4. *p*-Nitrobenzoyl-α-aminobutyrate	4-Nitrobenzoyl-α-aminobutanoate	271	122	
5. *p*-Aminobenzoyl-γ-aminobutyrate	4-Aminobenzoyl-γ-aminobutanoate	271	122	
6. *p*-Aminobenzoyl-α-aminovalerate	4-Aminobenzoyl-α-aminopentanoate	271	122	

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of		
		Active Secretion		Reabsorption
		In vivo	*In vitro*	
7. *p*-Aminobenzoyl-δ-amino-valerate	4-Aminobenzoyl-δ-amino-pentanoate	271	122(?)	
8. *p*-Nitrobenzoyl-δ-amino-valerate	4-Nitrobenzoyl-δ-amino-pentanoate	274	122	
9. 1,4-(bis-4-aminobenzoyl) ornithine	1,4-(bis-4-aminobenzoyl)-α,δ-diaminopentanoate		122	
10. *p*-Aminobenzoyl-ε-amino-caproate	4-Aminobenzoyl-ε-amino-hexanoate	274		
11. *p*-Aminobenzoylsarcosine	4-Aminobenzoylmethyl-aminoacetate	274, 466		
12. *p*-Nitrobenzoylsarcosine	4-Nitrobenzoylmethylamino-acetate	271		
13. *p*-Acetamidobenzoylsarcosine	4-Acetamidobenzoylmethylaminoacetate	466		
14. 1-Aminobenzoyl-α-amino-phenylacetate	1-Aminobenzoyl-α-amino-phenylacetate	271		271
15. N-(4-Aminobenzoyl)-glutamate	N-(4-Aminobenzoyl)-glutaminate			
16. NAP-Taurine	N-(4-Azido-2-nitro-phenyl)-2-aminoethyl-sulfonate	461		
17. Taurocholate	2-[[3α,7α,12α-Trihydroxy-24-oxo-5β-cholan-24-yl] amino]ethanesulfonate	542*	17	19, 491§, 505, 542
18. 2,4-Dichlorophenoxyacetate taurine conjugate	2-[2,4-Dichlorophenoxy-ethanoate]ethanesulfonate			
19. Mercapturic acid	S-benzyl-N-acetyl-2-amino-3-mercaptopropanoic acid	237		
C. Glucuronides				
1. Menthylglucuronide	D-Glucuronic acid 5 methyl-2-(1-methyl-ethyl)cyclohexaneether	452		
2. Phenylglucuronide	D-Glucuronic acid phenolether	452		
3. Resorcinylglucuronide	D-Glucuronic acid 1,3-benzenediol monoether	452		
4. Phlorizinglucuronide	D-Glucuronic acid 1-[2-(β-D-glucopyranosyloxy)-4,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)-1-propanone ether	63		
5. Catecholglucuronide	D-Glucuronic acid 1,2-benzenediol ester	383		
6. Estriolglucuronide	D-Glucuronic acid estra-1,-3,5(10)-triene-3,16α,17β-triol	68		
7. Estroneglucuronide	D-Glucuronic acid 3-hydroxy estra-1,3,5(10)-trien-17-one ether	68		68
8. Estradioleglucuronide	D-Glucuronic acid estra-1,-3,5(10)-triene-3,17α-diol ether	68		68
9. Aldosteronglucuronide	D-Glucuronic acid 11β,21-dihydroxy-3,20-dioxo-pregn.-en-18-al	178		178
10. Salicylacylglucuronide	D-Glucuronic acid 2-hydroxybenzoyl ester	406		
11. Salicylphenolic glucuronide	D-Glucuronic acid 2-carboxyhydroxybenzene ether	406		
12. *o*-, *m*-, *p*-Aminobenzoyl-	D-Glucuronic acid 2-, 3-, 4-	272	272	

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of		
		Active Secretion		Reabsorption
		In vivo	*In vitro*	
glucuronide	aminobenzoate ester			
13. Benzo(a)pyrene-7,8-diol-glucuronide	D-Glucuronic acid 7,8-dihydroxybenzo(a)pyrene	366		
D. Sulfates				
1. Phenolsulfuric ester	Phenolsulfuric ester	452		
2. Resorcinolsulfuric ester	1,3-Benzenediolsulfuric ester	452		
3. Catecholsulfuric ester	1,2-Benzenediolsulfuric ester	383		
4. Morphinesulfuric ester	7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diolsulfuric ester	310, 498		
5. Paracetamolsulfuric ester	N-(4-Hydroxyphenyl)-acetamidesulfuric ester	136		136
6. Benzo(a)pyrene-7,8-diol sulfuric ester	7,8-Dihydroxybenzo(a)-pyrene sulfuric ester	366		
II. Benzoate derivatives				
1. Salicylate	2-Hydroxybenzoate	103, 203, 390, 406, 508, 519		103, 203, 390, 406, 508, 519
2. *m*-Hydroxybenzoate	3-Hydroxybenzoate	311, 312		311, 312§
3. *p*-Hydroxybenzoate	4-Hydroxybenzoate	504		504
4. *p*-Aminobenzoate	4-Aminobenzoate	297		
5. *o*-, *m*-, *p*-Acetamidobenzoate	2-, 3-, 4-Acetamidobenzoate	273	122	
6. Gentisate	2,5-Dihydroxybenzoate	503		503
7. Diflunisal	2′,4′-Difluoro-4-hydroxy-[1,1′-biphenyl]-3-carboxylate	7*		7
8. *m*-, *p*-Nitrobenzoate	3-, 4-Nitrobenzoate	503		
9. *o*-Nitrobenzoate	2-Nitrobenzoate	503		
10. *m*-, *p*-Nitrosalicylate	3-, 4-Nitro-2-hydroxybenzoate	503		
11. *p*-Aminosalicylate	4-Amino-2-hydroxybenzoate	51, 235		235
12. *m*-, *p*-Acetamidobenzoate	3-, 4-Acetamidobenzoate	273	122, 272	
13. *o*-Acetamidobenzoate	2-Acetamidobenzoate	273	272	273
14. 4-Acetamido-3,5-diiodobenzoate	4-Acetamido-3,5-diiodobenzoate	270, 273	122, 270	
15. 3-Amino-2,4,6-triiodobenzoate	3-Amino-2,4,6-triiodobenzoate	270	122, 270	
16. 3-Acetamido-2,4,6-triiodobenzoate	3-Acetamido-2,4,6-triiodobenzoate	270, 273	270	
17. 3,5-Diacetamido-2,4,6-triiodobenzoate	3,5-Diacetamido-2,4,6-triiodobenzoate	270, 273	270	
18. 3,5-Diproprionamido-2,4,6-triiodobenzoate	3,5-Diproprionamido-2,4,6-triiodobenzoate	270	122, 270	
19. *p*-Dimethylsulfamylbenzoate	4-[(Dimethylamino)-sulfonyl]benzoate		432	351, 509
20. *p*-Diethylsulfamylbenzoate	4-[(Diethylamino)-sulfonyl]benzoate		432	351, 509
21. Probenecid	4-[(Dipropylamino)-sulfonyl]benzoate	114, 509	31, 62, 431	44, 509
22. 2-Nitroprobenecid	4-[(Dipropylamino)-sulfonyl]-2-nitrobenzoate	503		
23. 2-Hydroxyprobenecid	4-[(Dipropylamino)-sulfonyl]-2-hydroxybenzoate	47		
24. 2-Chloroprobenecid	4-[(Dipropylamino)-sulfonyl]-2-chlorobenzoate	47		

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of Active Secretion *In vivo*	Demonstration of Active Secretion *In vitro*	Demonstration of Reabsorption
25. *p*-Dibutylsulfamylbenzoate	4-[(Dibutylamino)sulfonyl]-benzoate		432	351, 509
26. *p*-Alkyl(C_1–C_6)sulfamylbenzoate	4-[(Alkyl(C_1–C_6)amino)-sulfonyl]benzoate		430	
27. Cyclohexylsulfamylbenzoate	4-[(Cyclohexylamino)-sulfonyl]benzoate		421	
28. Cycloheptylsulfamylbenzoate	4-[(Cycloheptylamino)-sulfonyl]benzoate		421	
29. Piperidylsulfamylbenzoate	4-[(Piperidylamino)-sulfonyl]benzoate		422	
30. Benzylsulfamylbenzoate	4-[(Phenylmethylamino)-sulfonyl]benzoate		422	
31. Carinamide	4-[[(Phenylmethyl)-sulfonyl]amino]benzoate	41, 139, 503	126	41, 503
32. Furosemide	5-(Aminosulfonyl)4-chloro-2-[(2-furanylmethyl)-amino]benzoate	59, 115, 222, 338, 339	218	
33. Bumethanide	3-(Aminosulfonyl)-5-(butylamino)-4-phenoxybenzoate	339		339
34. Piretanide	4-Phenoxy-3-(1-pyrrolidinyl)-5-sulfamoylbenzoate	339		339
35. Diatrizoate	3,5-Bis(acetylamino)-2,4,6-triiodobenzoate	324	324	324
36. Iodipamide	3,3′-[(1,6-Dioxo-1,6-hexanediyl)diimino•s[2,4,6-triiodobenzoate]	38	14, 15	
37. Fluorescein	3′,6′-Dihydroxyspiro]isobenzofuran-1(3H),9′-[9H]xanthen]-3-one		66, 67, 106, 332, 333	
III. Acetate and proprionate derivatives				
1. 5-Hydroxyindolacetate	Indole-5-hydroxy-3-ethanoate	129, 206, 314a	122	
2. 2-Pyridone-N-acetate	2-Oxo-1(4H)-pyridineethanoate	444		
3. Diodrast	3,5-Diiodo-4-oxo-1(4H)-pyridineethanoate	145, 243, 444		253, 257, 471¶
4. Phenoxyacetate	Phenoxyethanoate	147, 503		503
5. 2,4-Dichlorophenoxyacetate	2,4-Dichlorophenoxy-ethanoate	147, 276a, 367	37, 367	
6. 2,4,5-Trichlorophenoxyacetate	2,4,5-Trichlorophenoxy-ethanoate	275	37, 227	
7. 4-Chlorophenoxyacetate	4-Chlorophenoxyethanoate	147		
8. 2,2-Bis(*p*-cholorophenyl)-acetate	2,2-Bis(4-chlorophenyl)-ethanoate	368	364, 365, 368	
9. Tienilate	2,3-Dichloro-4-(2-thienylcarbonyl)phenoxy-ethanoate	340, 445		
10. Indomethacin	1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate	440		340, 445
11. Ethacrynic acid	[2,3-Dichloro-4-(2-methylene-1-oxobutyl)-phenoxy]acetate	43, 196, 339	87, 199	
12. MK-196	(6,7-Dichloro-2-methyl-1-oxo-2-phenyl-5-indanyloxy)acetate	156, 339		156
13. Ozolinone	1-Methyl-2-one-3-(N-piperidinyl)-4-ethanoate	193, 194		

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of		
		Active Secretion		Reabsorption
		In vivo	*In vitro*	
14. Mersalyl	[3-[[2-(Carboxymethoxy)-benzoyl]amino]-2-methoxypropyl]hydroxy-mercury salt	80, 82		
15. Halofenate	4-Chloro-α-[3-(trifluoromethyl)phenoxy]benzeneethanoate			
16. Zomepirac	5-(4-Chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-ethanoate	343†		
IV. Heterocyclic carboxylates				
1. 5-Aminoorotate	5-Amino-1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylate		122	
2. Pyrazinoate	Pyrazinecarboxylate	157, 327, 507		157, 327§, 507
3. 5-Hydroxypyrazinoate	5-Hydroxypyrazinecarboxylate	507		
4. Benzylpenicillin	3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo-[3.2.0.]heptane-2-carboxylate	21, 45, 137, 374		
5. Amylpenicillin	*n*-Amylpenicillinate	137		
6. Ampicillin	α-Aminobenzylpenicillin	142†		
7. Ancillin	6-(2-Phenylbenzamido)-penicillanate	401†		
8. Nafcillin	6-(2-Ethoxyl-1-naphthamido)penicillanate	21		
9. Cephaloridin	(6R-trans)-1-[[2-carboxy-8-oxo-7-[(2-thienylacetyl)-amino]-5-thia-1-azabicyclo[4.2.0.]oct-2-en-3-yl]-methyl]pyridinium		476, 479	
10. Cephalothin	7-(2-Thienylacetamido)-cephalosporanate	21, 483		
11. 3-Hydroxy-2-phenylcinchoninate	3-Hydroxy-2-phenyl-4-quinolinecarboxylate	503		
V. Sulfonamides				
1. N^4-Acetylsulfamerazine	4-(Acetylamino)-N-(4-methyl-2-pyrimidinyl)-benzenesulfonamide	138	126	
2. N^4-Acetylsulfamethazine	4-(Acetylamino)-N-(4,6-dimethyl-2-pyrimidinyl)-benzenesulfonamide		126	
3. N^4-Acetylsulfathiazole	4-(Acetylamino)-N-2-thiazolylbenzenesulfonamide		126	
4. Sulfathiazole	4-Amino-N-2-thiazolylbenzenesulfonamide	297		
5. N^4-Acetylsulfanilylsulfanilamide	4-Sulfamoylsulfanilanilide	417	126	
6. Sulfisoxazole	4-Amino-N-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide	5, 346	126	5, 345
7. N^4-Acetylsulfisoxazole	4-(Aminoacetyl)-N-(3,4-dimethyl-5-isoxazolyl)-benzenesulfonamide		126	
8. Sulfamethizole	4-Amino-N-(5-methyl-1,-3,4-thiadiazol-2-yl)-benzene sulfonamide	297, 346		
9. Sulfamethylthiazole	4-Amino-N-(4-methyl-2-thiazole)benzenesulfonamide		126	

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of Active Secretion *In vivo*	Demonstration of Active Secretion *In vitro*	Demonstration of Reabsorption
10. 2-Benzenesulfonamido-1,-3,4-thiadiazole-5-sulfonamide	2-Benzenesulfonamide-1,-3,4-thiadiazole-5-sulfonamide	474		
11. *o*-, *m*-, p-Sulfanilamidobenzoate	2-, 3-, 4-Sulfanilamidobenzoate	417	126	
12. *o*-, *p*-Sulfanilamidophthalate	2-, 4-Sulfanilamidophthalate	417	126	
13. *m*-, *p*-Sulfanilamidosulfonate	3-, 4-Sulfanilamidosulfonate	417	126	
14. Sulfanilglycin	Sulfanil aminoacetate	417	126	
15. N^1-Acetylsulfanilamide	4-Amino-N-acetylbenzenesulfonamide	41	126	
16. N^1-Acetylsulfisoxazole	4-Amino-N-acetyl(3,4-dimethyl-5-isoxazolyl)-benzenesulfonamide	41	126	
17. N^1-(Toluenesulfonyl)-sulfanilamide	4-Amino-N-(toluenesulfonyl)benzenesulfonamide		126	
18. Acetazolamide	N-[5-(Aminosulfonyl)-1,-3,4-thiadiazol-2-yl]acetamide	508		
19. Carzenide	4-Carboxybenzenesulfonamide	41	126	
20. Chlorothiazide	6-Chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	8, 42, 339, 414		
21. Hydrochlorothiazide	6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	42, 337, 414	135	
22. Trichhlormethiazide	6-Chloro-3(dichhloromethyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	42	135	
23. Benzthiazide	6-Chloro-3[[(phenylmethyl)thio]methyl]-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	503		
24. Benzydroflumethiazide	3,4-Dihydro-3-(phenylmethyl)-6-(trifluoromethyl)-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide	356		
25. Saccharin	1,2-Benzisothiazol-3(2H)-one 1,1-dioxide	57		
26. Cyclopenthiazide	6-Chloro-3-(cyclopentylmethyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide		135	
VI. Other heterocyclic compounds (charge on ring)				
1. Phenylbutazone	4-Butyl-1,2-diphenyl-3,5-pyrazolidinedione	198, 503		198, 503
2. Sulfinpyrazone	1,2-Diphenyl-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione	198, 354, 503		198, 354, 503
3. *p*-Hydroxysulfinpyrazone	1,2-Diphenyl-4-[2-(4-hydroxyphenylsulfinyl)-ethyl]-3,5-pyrazolidinedione	354		354
4. Urate	7,9-Dihydro-IH-purine-2,-6,8(3H)-trione	203, 362, 363	109, 154‡, 357, 409, 426	

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of Active Secretion *In vivo*	Demonstration of Active Secretion *In vitro*	Demonstration of Reabsorption
5. Xanthine	3,7-Dihydro-IH-purine-2,6-dione	6	34	6§
6. 2,4-Dinitrophenol	2,4-Dinitrophenol		36	
7. Nitrofurantoin	1-[[(5-Nitro-2-furanyl)-methylene]amino]-2,4-imidazolidinedione	350	2	350
VII. Sulfonates				
1. Phenol red	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bisphenol S,S-dioxide	302, 335, 416	86, 167, 168, 170, 224	183
2. Chlorphenol red	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bis[2,6-dichlorophenol]S,S-dioxide	454, 504	122, 168, 254, 419	504
3. Bromphenol blue	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bis[2,6-dibromophenol]S,S-dioxide	183, 454	168, 419	183
4. Bromcresol green	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bis]2,6-dibromo-3-methylphenol]S,S-dioxide	183, 454	122, 226, 419	
5. Bromcresol purple	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bis[2-bromo-6-methylphenol]S,S-dioxide			
6. Bromthymol blue	4,4′-(3H-2,1-Benzoxathiol-3-ylidene)bis[2-bromo-3-methyl-6-(1-methylethyl)phenol]S,S-dioxide	183, 454		183
7. Indigo Carmine	2-(1,3-Dihydro-3-oxo-5-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonate	247	122	
8. Ponceau 3R	3-Hydroxy-4-[(2,4,5-trimethylphenyl)azo]-2,7-naphthalene disulfonate		122	
9. Tropaeolin O	4-[(2,4-Dihydroxyphenyl)azo]benzenesulfonate		122	
10. Tropaeolin OO	4-[[4-(phenylamino)-phenyl]azo]benzenesulfonate		122	
11. 1-Naphthylamine-4-sulfonate	4-Amino-1-naphthalenesulfonate		122	
12. 2-Aminonaphthalene 3,6-disulfonate	2-Amino-3,6-naphthalene disulfonate		122	
13. 2,7-Naphthalenedisulfonate	2,7-Naphthalenedisulfonate	329		
14. 1,5-Naphthalenedisulfonate	1,5-Napththalenedisulfonate	329		
15. 4-Acetamidobenzenesulfonate	4-(Acetylamino)benzenesulfonate		122	
16. Iodomethanesulfonate	Iodomethanesulfonate	444		
17. DIDS	4,4′-Diisothiocyano-2,2′-stilbenedisulphonate	277		
18. Amaranth	3-Hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-2,7-naphthalenesulfonate		124	
19. Tartrazine	4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-1H-pyrazole-3-carboxylate		124	

TABLE 1—*Continued*

Organic Anions	Chemical Names	Demonstration of Active Secretion *In vivo*	Demonstration of Active Secretion *In vitro*	Demonstration of Reabsorption
VIII. Amino acids and derivatives				
1. L-Tyrosine	L-Tyrosine	522		232, 522
2. 3,5-Dichloro-L-tyrosine	3,5-Dichloro-L-tyrosine	232		232
3. 3,5-Dibromo-L-tyrosine	3,5-Dibromo-L-tyrosine	232		232
4. 3,5-Dinitro-L-tyrosine	3,5-Dinitro-L-tyrosine	232		
5. 3,5-Diiodo-L-tyrosine	3,5-Diiodo-L-tyrosine	232, 233		
6. N-Acetyl-3,5-diiodotyrosine	N-Acetyl-3,5-diiodotyrosine	233		
7. Di-acetyl-3,5-diiodotyrosine	Di-acetyl-3,5-diiodotyrosine	233		
8. 3,5-Diiodo-*o*-methyltyrosine	3,5-Diiodo-2-methyltyrosine	273		
9. L-Tryptophane	L-Tryptophane	521*, 522		521, 522
10. D-Tryptophane	D-Tryptophane	521		
11. N-Acetyltryptophane	N-Acetyltryptophane	521	521	
12. L-Phenylalanine	L-Phenylalanine	522		434, 522
13. 3-(*p*-Hydroxyphenyl)-proprionate	3-(4-Hydroxyphenyl)-proprionate	522		522
14. Pantothenate	(R)-N-(2,4-Dihydroxy-3,3-dimethyl-1-oxobutyl-β-alanine	50, 139, 395		395, 529
15. Methotrexate	L-(+)N-[P[[(2,4-Diamino-6-pteridinyl)methyl]-methylamino]benzyl]-glutamate	236	236	236
16. Folic acid	N-[4-[[(2-Amino-1,4-dihydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamate	523		
IX. Miscellaneous compounds				
1. Prostaglandin $F_{2\alpha}$	7-[3,5-Dihydroxy-2(3-hydroxy-1-octenyl)-cyclopentyl]-5-heptenoate	46, 379		
2. Prostaglandin E_2	7-[3-Hydroxy-2-(3-hydroxy-1-octenyl)-5-oxo-cyclopentyl]-5-heptenoate	379	238‡	
3. Ascorbate	3-Oxo-L-gulofuranolactone	266, 386		266, 539§
4. Cholate	3α,7α,12α-Trihydroxy-5β-cholan-24-oate		17	491, 505
5. Deoxycholate	3α,12α-Dihydroxy-5β-cholan-24-oate		17	
6. Chenodexoycholate	3α,7α-Dihydroxy-5β-cholan-24-oate		17	
7. Oxalate	Ethanedioate	85, 191, 268, 520		191, 268
8. Thiosulfate	Thiosulfate			69, 141, 285, 323, 490
9. Creatinine	2-Amino-1,5-dihydro-1-methyl-4H-imidazol-4-one	4, 69, 101, 336, 377, 412, 465		69, 284, 336
10. Urea (frogs)	Carbamide	165		
11. Allantoin	(2,5-Dioxo-4-imidazolidinyl)urea	387, 408	387, 408	387, 408
12. cAMP	Adenosine 3′,5′-(hydrogen phosphate)	100		
13. cGMP	Guanosine 3′,5′-(hydrogen phosphate)	100		
14. Riboflavin	7,8-dimethyl-10-(D-ribo-2,-3,4,5-tetrahydroxypentyl)isoalloxazine)		449	

The criteria used for categorizing compounds as substrates of the renal organic anion secretion system are: 1) In vivo—Inhibition of urinary excretion by probenecid, PAH or other representative members of the system, in conjunction with demonstration of net secretion, or excess ipsilateral excretion by the Sperber technique (in the chicken). In a few cases net secretion has not been demonstrated, either because of predominant reabsorption (*) or by the lack of appropriate clearance data (†). 2) In vitro—Demonstration of O_2 metabolism-dependent

nique include the semiquantitative nature of the measurements. The rate of uptake is much lower than tubular secretion rates observed in vivo, and the level of accumulation obtained cannot be related to rates of transtubular transport. This is demonstrated for cephaloridine (479) and bile acids (17), which are accumulated to high levels in kidney slices, but for which tubular secretion in vivo is minimal (480, 541), presumably because of restricted transport from cell to tubule fluid across the luminal membrane. Secondly, intracellular accumulation per se does not constitute any proof that intracellular accumulation occurs in a secretory direction, i.e. by transport across the basolateral rather than across the luminal membrane. For instance various amino acids (385, 397) and monohexoses (264, 265), which are reabsorbed by the kidney, have been shown to be accumulated against concentration gradients by cortical slices. It is conceivable that probenecid could inhibit intracellular accumulation of an organic anion across the luminal membrane, i.e. transport in the reabsorptive direction. This possibility must be borne in mind, although in most instances it appears that reabsorption of organic anions is not inhibited by representative members of the organic anion secretory system (section IV D). Finally, complications are also introduced by the use of transported substrates which are passively bound by tissue components. In this case accumulation must be compared in the aerobic and anaerobic state (418).

B. SEPARATED RENAL TUBULES. Some of the drawbacks of the kidney slice technique for quantitative studies can be avoided by the use of separated tubule fragments, prepared by the action of collagenase on minced cortex tissue (75). An improved method for preparation of separated tubules has recently been described, which incorporates a Ficoll gradient centrifugation step to remove nonviable material (10). The separation of tubule segments from one another facilitates mixing of added compound throughout the entire system. In comparison to kidney slices the tubule fragments are capable of accumulating PAH much more quickly (77, 425) and have a higher oxygen consumption and ATP content (10). On the other hand such preparations are much more fragile than kidney slices, e.g. they cannot withstand incubation in Na^+-free incubation media (359).

C. ISOLATED, PERFUSED KIDNEY. Techniques for isolation and perfusion of rat kidney with retention of good functional properties have been described (60, 398). Although the technique has not been used extensively it combines the advantage of in vitro techniques with the possibility of measuring transtubular transport rates, while at the same time permitting an evaluation of the physiological status of the preparation by conventional clearance measurements. Cellular uptake across the basolateral membrane may be studied under nonfiltering conditions by inclusion of a high concentration of macromolecule in the perfusion fluid, e.g. 10% dextran (475). The technique has been used to examine the effect of protein binding on excretion of furosemide (59) and phenol red (335), and to study the cellular uptake of octanoate across the luminal and basolateral membrane (475).

D. PERFUSION OF ISOLATED TUBULES. Techniques for perfusion studies of single, isolated tubules were pioneered by Burg and co-workers (73). Long tubule fragments are dissected from renal tubules of rabbit kidney under microscopic guidance. The two ends of an isolated tubule segment are connected with a micropipette for perfusion at one end and collection of tubule fluid from the other end. Reabsorption of a substance is studied by adding it to the perfusate and measuring the concentration after passage through the tubular lumen. To correct for fluid absorption, inulin is added to the perfusate to estimate fluid absorption. Secretory rates are measured by immersing the tubule fragment in a medium that contains the substance in question and then perfusing the tubule lumen in the absence of the substance. Procedures have also been developed to measure the concentration of substance inside the renal cells after filling the tubule lumen with an inert oil (73, 76) or shortly after the end of the perfusion (18). This enables flux rates to be calculated across both the basal and luminal cell membranes (478). Concerning the viability of the preparations, Burg and Orloff (76) have reported that Na^+ and fluid reabsorption occur to the same extent as measured by micropuncture in vivo.

In addition to the possibility of measuring details of transcellular transport, a major advantage of the technique is to study the function of tubule segments, which are not accessible to micropuncture.

E. ISOLATED PLASMA MEMBRANE VESICLES. A development of great importance in the study of transport processes at the subcellular level is the use of the isolated membrane vesicles. Separation of vesicles derived from the basolateral and luminal membranes of the rat kidney has been achieved by the application of freeflow electrophoresis to a plasma membrane fraction prepared from sucrose homogenates of kidney cortex by differential centrifugation (210, 384). The free-flow electrophoresis

accumulation of compound by renal tissue that is inhibited by probenecid, PAH, or other representative members of the system. The suitability of these criteria is discussed in section I C. The column includes data on demonstration of transtubular transport by microperfusion of isolated tubule fragments (‡). 3) Reabsorption—The criterium for reabsorption is demonstration of a smaller clearance or less accumulation in tubular fluid than that of a glomerular reference substance (inulin). §, Cases where there is evidence for carrier-mediated reabsorption in various species, either by clearance data or by studies on vesicles of luminal membranes; ¶, carrier-mediated reabsorption in the frog.

Except for the very thoroughly studied compounds (urate, PAH, phenolsulfulfophthalein dyes, diodrast), the majority of studies are limited to the following species: dog, man, rabbit, rat, and chicken. There is at present little evidence for marked differences in tubular secretion among these species, except for urate (section II B) and the x-ray contrast compounds, iodipamide and diatrizoate, where secretion has been demonstrated in the rabbit and shown to be absent in the dog (77–80).

method has not given satisfactory results in other species. Another method includes treatment with Mn^{++}, Ca^{++}, and Mg^{++} to preferentially sediment the basolateral membranes by centrifugation, followed by gradient centrifugation on sucrose (252). By comparative studies of the transport properties of luminal and basolateral membrane preparations it is often possible to clarify the role that each membrane system plays for transcellular transport. Active transport driven by inorganic electrolyte gradients (Na^+) is easily detected as a transient accumulation of the compound in the intravesicular space. Similarly, the importance of the transmembranal potential difference for transport may be studied by the judicious use of electrolyte gradients and ionophores. Characterization of the transport process in terms of common substrates (competitive inhibition) and affinity can be performed. Finally, such preparations provide an opportunity to study binding of substrates to the carrier system (in leaky preparations) which may eventually lead to isolation of the transport system (220). Disadvantages of the technique are that the yield of membranes for the experiments is quite small, and a procedure that gives consistently good preparations of basolateral membranes has been difficult to establish (in contrast to luminal membrane preparations). Furthermore, difficulties in interpretation may arise because of variations in the polarity of the vesicles (inside-out or outside-in).

F. CULTURED RENAL CELLS. The ability of epithelial cells to form a continuous sheet of a unicellular membrane when grown on a firm support in many cases permits transport study on a geometrically simple system. The status of the field as applied to renal cells has been reviewed by Handler et al. (207). Most studies until now have been performed on the MDCK cell line, but cell cultures with more differentiated function are being developed.

2. In Vivo Techniques. Standard clearance techniques, with inulin as a marker of glomerular filtration rate, can be relied upon to give precise information on the overall tubular handling of organic anions. However, many organic anions are simultaneously reabsorbed by the tubules, which can make it difficult to uncover the characteristics of the secretory process, unless simplifying assumptions are made and excretion is studied under a variety of conditions. An example of an analysis of this kind is shown in figure 1. Other examples may be found in work by Weiner et al. (509) and Møller (319).

In the Sperber (450) technique, advantage is taken of the double blood supply of the chicken kidney to study the existence of tubular secretion by infusion of the test substance into the renal portal system via the saphenous vein. The ipsilateral excretion of the infused compound in excess of that of the other kidney may then be attributed to tubular secretion. A recent improvement includes ligation of vessels that partially shunt the blood supply away from the perfused kidney (337).

The micropuncture methodology, including microperfusion and microinjection, plays an important role in defining sites of tubular secretion and reabsorption, as in other fields of renal study. Most data have been obtained during free-flow (389). Microperfusion was used by Deetjen and Sonnenberg (116) to study the kinetics of PAH transport. Recently, peritubular capillaries and tubule lumens were perfused simultaneously to study the secretory flux of urate in the rat (514), a species in which it is difficult to study tubular secretion because of net reabsorption of urate. A technically less demanding, but also less precise method than micropuncture for localization is the stop-flow technique [for a review see (298)]. In this case it is also often advantageous to use the method for flux measurements by injection of the organic anion during the stop-flow period (542).

The dynamics of tubular transport may be studied by injection of organic anion into a renal artery and rapid, sequential sampling of blood or urine fractions from the renal vein or ureter (172, 435). The concentration of the test substance, relative to that of an extracellular marker (inulin), is used to assess transport characteristics across the basolateral and luminal membranes, after appropriate corrections for recirculation by measurements on the contralateral kidney.

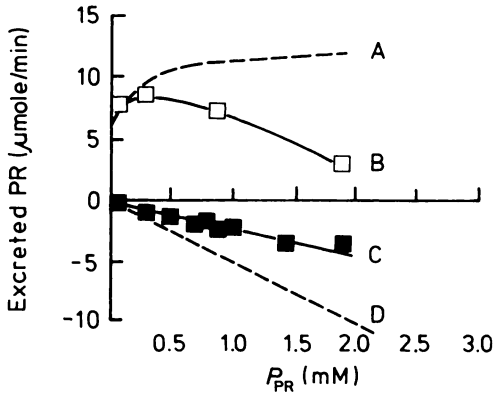

FIG. 1. Approximative analysis of phenol red secretion and reabsorption in the rabbit as a function of ultrafiltrable dye in the plasma. Phenol red is assumed to be secreted by a saturable mechanism (upper broken line, curve A), but net secretion is found to be decreased at a high plasma concentration (curve B), probably as the result of concomitant reabsorption (curve D). The reabsorptive process seems to have diffusional characteristics as evidenced by proportionality between net reabsorption and ultrafiltrable phenol red in plasma after inhibition of secretion with 150 mg probenecid/kg body weight (curve C). The lower reabsorptive rate observed after administration of probenecid than that calculated from the net secretion curve may either be due to incomplete blockade of secretion, or partial inhibition of reabsorption by probenecid, or to a smaller concentration of phenol red in tubule fluid after inhibition of secretion. Adapted from Gerdes et al. (183).

II. Renal Handling of Some Selected Organic Anions

A. p-Aminohippurate (PAH)

The preference by investigators to use PAH as a prototype with which to study the properties of the organic anion system stems from several properties of the compound: It is efficiently secreted and undergoes limited

metabolic alteration and reabsorption in most species. It is only weakly bound by plasma proteins, and is easy to determine chemically. However, it should be noted that in most species, except rabbit, the amino group is acetylated to a significant extent in the kidney-slice system (382, 415) and also in clearance experiments in the pig (204). Furthermore, PAH is reabsorbed by an active mechanism in Necturus (253).

1. Tubular Localization of Secretion. There is general agreement that secretion of PAH as well as of other organic anions is confined to the proximal tubule of the mammalian kidney. For PAH, the evidence has been obtained by micropuncture (98), by microperfusion in vivo (9, 116, 470), and by in vitro studies (8, 478, 528). In microdissected fragments of proximal tubules from the rabbit, Tune et al. (478) found that the highest rates of secretion and intracellular accumulation of PAH occurred in pars recta. At high PAH concentrations the secretory rates were so large that net fluid secretion into the lumen of pars recta tubules occurred, because of the osmotic effect of the secreted PAH (188). As pointed out by Woodhall et al. (528), a more satisfactory subdivision of functional capacity of the proximal tubule in three segments, S_1 to S_3, is obtained by the application of morphological differences at the cellular level (303, 304). In the microperfusion study by Woodhall et al. (528), high secretion rates of PAH in the rabbit kidney were exclusively associated with the S_2-segment, which extends from the end of the convoluted tubule and through pars recta to the corticomedullary zone. The exact anatomical location of PAH secretion has not been determined with the same precision in other species than the rabbit. The excretion pattern in the rat kidney may be similar, since in this species no evidence for net secretion of PAH in the early proximal tubule was obtained in micropuncture experiments (512). However, Roch-Ramel et al. (393) have reported that in similar free-flow micropuncture experiments performed in the pig the pars convoluta is the predominant site of PAH secretion. It, therefore, seems improbable that the morphology of renal cells per se is directly related to the capacity for organic anion secretion. It may be that differentiation of secretory function within the proximal tubule can be considered an example of neutral mutation occurring during phylogenesis.

2. Steps of Transtubular Transport of PAH. Tune et al. (478), in their microperfusion experiments on isolated tubule segments, found that the amount of PAH transported from the suspension medium into the tubule lumen was nearly constant over a wide range of perfusion rates; this suggests that reabsorption is negligible as compared to the secretory flux. Tubular secretion was accompanied by intracellular accumulation and was inhibited by probenecid. The intracellular PAH concentrations were much higher than those in the tubule fluid. At zero perfusion rate intracellular accumulation reached values of around 140 in the pars recta and around 25 in the pars convoluta cells. Microperfusion studies on the excretion of PAH by the proximal tubules of an amphibian species (Thamnophis) have confirmed the presence of an active step localized at the basolateral membrane (107, 108). The results were analyzed according to the model shown in figure 2, in which it is assumed that active transport only occurs across the basolateral membrane, while passage across the luminal membrane occurs by diffusion with equal permeability constants for influx and efflux. The data indicate that efflux occurs much more rapidly across the luminal than the basolateral membrane, resulting in an efficient transport of PAH from the medium to tubule lumen.

The data of figure 2 refer to the situation at a low medium concentration of PAH (2×10^{-5} M). Dantzler (107) found that a maximal level of PAH secretion was attained at a medium concentration of 6×10^{-5} M PAH. A similar observation was made by Schäli and Roch-Ramel (409) in microperfusion of pars recta of rabbit proximal tubules, but the maximal level of secretion was higher (2000 compared with 300 fmol of PAH secreted/min/mm of tubule). Even higher values for secretion were obtained by Shimomura et al. (433) in the S_2 segment of the rabbit proximal tubules (7430 ± 1340 fmol/mm/min). In the latter study the K_m value of all three segments for half-maximal PAH transport was similar (0.113 to 0.195 mM), despite much lower maximal secretory rates in S_1 and S_2 segments. The authors suggest that segmental differences may be attributable to different densities of PAH carriers with the same properties along the proximal tubule.

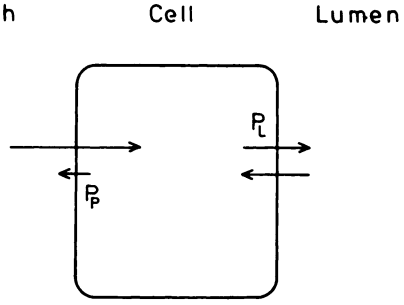

FIG. 2. Model used for analysis of tubular secretion of PAH across the basolateral and luminal membrane. Uptake from bath to cell is active and leads to intracellular accumulation of PAH. Backleakage of PAH across the basolateral membrane (characterized by an apparent permeability coefficient P_P) is small, relative to both bath to cell uptake and transfer across the luminal membrane. This accounts for the independence of tubular secretion of PAH on tubule fluid flow. In the model the apparent permeability coefficient for transfer across the luminal membrane (P_L) is assumed to be of equal magnitude in both directions. From the study of Tune et al. (478) $P_L = 59 \times 10^{-6}$ cm/sec and $P_P = 3.7 \times 10^{-6}$ cm/sec for pars recta of rabbit kidney at 37°C and 2×10^{-5} M PAH in medium, calculated on the basis of the concentration difference between cell and bath or cell and tubule fluid respectively, by using data from both perfused and nonperfused tubules, and assuming that the surface area of the membranes can be calculated as smooth areas from tubule diameter. By the same procedure Dantzler (107) found $P_L = 35 \times 10^{-6}$ cm/sec and $P_P = 11.7 \times 10^{-6}$ cm/sec in snake tubules. A somewhat lower value was found (5×10^{-6} cm/sec) when P_P was measured in a more direct way by efflux from tubules with oil filled lumen (107).

Saturation in the microperfusion studies occurs at a lower concentration than that which is required to attain a half-maximal uptake rate of PAH (approx. 0.5 mM) in separated renal tubule fragments (234, 423, 425), basolateral membrane vesicles (39, 252), and rabbit kidney slices (184, 348, 359, 365). This may suggest a relative change in the apparent permeability coefficients across the two membranes as a function of the medium concentration: At low bath concentrations (2×10^{-5} M) where $P_p \ll P_L$ (fig. 2), cellular uptake across the basolateral membrane virtually alone determines the rate of tubular secretion of PAH. However, if at a higher PAH concentration the luminal membrane becomes less permeable to the organic anion, this would tend toward earlier saturation of the tubular secretory rate as a function of bath concentration than would be observed for uptake across the basolateral membrane alone. It is obvious that this view entails that transfer across the luminal membrane does not, in fact, occur by diffusion, but by mediated transport. The likelihood of this possibility is considered at the end of this chapter.

The schematic model of PAH transport evolving from studies on perfused fragments of isolated proximal tubules is challenged to some extent by micropuncture experiments in the rat kidney in vivo. A reduction of PAH secretion was reported to occur at a high intraluminal concentration of PAH [Deetjen and Sonnenberg (116)] and by a reduction in the tubule perfusion rate, induced by changes in glomular filtration (209). However, this observation was not confirmed in free-flow micropuncture studies by Tanner and Isenberg (470) in the same species. Although there is evidence of some back flow of PAH in microperfusion experiments (446) and microinjection experiments (9) this process probably cannot account for the reduced secretion of PAH at a low tubule fluid flow (209). Since tubular reabsorption of tubule fluid is also reduced under these conditions, this is consistent with the view that increases in the secretory rate of PAH are associated with an enhanced reabsorptive rate of Na^+, as previously suggested from studies in the perfused frog kidney (493, 494, 495). The role of Na^+ for active transport of PAH is considered below. Häberle (209, 209a) points out that in the microperfusion experiments of Tune et al. (478) Na^+ dependent tubule fluid reabsorption remained nearly constant, despite changes in perfusion rate.

3. PAH Accumulation by Kidney Slices and Tubule Suspensions. In the presence of a suitable metabolic substrate like acetate, pyruvate and lactate uptake of PAH is approximately doubled (2, 10, 306), ordinarily resulting in accumulation levels of 10 to 12. However, it is common experience that the level of accumulation is variable, which in part may be attributable to differences in slice thickness and the region of the cortex from which the slice is derived. The prevailing opinion is that the uptake predominantly represents intracellular accumulation, since the lumen of the proximal tubules in the kidney slice preparation probably is in a collapsed state [but for a different view, see Wedeen and Weiner (500)]. Kinetic studies indicate that PAH uptake occurs at a relatively rapid rate in the first minutes after exposure to PAH and that this phase is followed by a steady, but slow process which eventually leads to appreciable accumulation (173, 174, 399, 400, 516). A biphasic uptake pattern is also observed in suspensions of rabbit kidney tubules, and here a maximal accumulation is reached within a relatively short time period (75, 425). In the experiments of Sheikh and Møller (425), rapid uptake resulted in an accumulation of about 2.5 and slow uptake in an accumulation of around 7.5. The rate of PAH transport during the rapid phase was comparable to secretory rates of PAH in vivo, but the slow phase was not. An inverse sequence of events takes place after preloading of kidney slices (162, 400) and tubule suspensions (234, 425), with PAH and transfer to a medium not containing organic anions. Under these conditions run-out occurs rapidly during the first few minutes and is followed by a slow release phase.

The biphasic uptake of PAH in separated renal tubules can be accounted for by a two-compartment system (425), but the morphological basis for such a subdivision has not been determined. Various authors have measured the run out of accumulated PAH and other organic anions in the presence of competitive inhibitors (30, 96, 162, 223, 254, 256, 400). Typically, low concentrations of inhibitor enhance outflow, whereas high concentrations inhibit this process. Enhanced outflow may either be attributed to inhibition of reuptake (suggesting the presence of an unstirred layer at the transporting membranes which seems reasonable, in particular for kidney slices), or to countertransport, i.e. to acceleration of efflux by exchange when another substrate is present in the medium. Inhibition of run-out suggests a direct effect on efflux, indicating that this process, as well as influx, is carrier-mediated.

4. Effect of Na^+ and K on PAH Accumulation. Experimental evidence consistent with Na^+-K^+-dependent PAH transport in kidney slices has been reported by many authors. Early studies emphasized the role of K^+ for accumulation of PAH (175, 263, 468). Other evidence that suggests a requirement for K^+ comes from observations that maximal secretion rates of PAH by microperfused proximal tubules of the snake depend on the presence of K^+ in the incubation media (7, 10, 110).

Chung et al. (92) were the first to show that PAH accumulation in the kidney slice system is also affected by Na^+. Accumulation of PAH was almost completely abolished in the absence of Na^+ and after preincubation in a Na^+-free medium ("leaching"), which reduced tissue concentrations to about 10 mM Na^+. The effect of Na^+-depletion was reversible, and the rate of PAH uptake was approximately proportional to the medium concentration of Na^+ up to 30 mM (184, 185). It was reported that Na^+ enhances the maximal rate of PAH transport, while K^+ increases the affinity of the transport system for PAH in rabbit kidney slices (184, 358). However,

Kikuta and Hoshi (250) reported that Na^+ rather than K^+ increased the affinity of the transport system for PAH in the goldfish kidney.

The Na^+ dependency of organic anion secretion must be assumed to be a general phenomenon, both occurring in different species and with different organic anions (67, 111, 333). Podevin et al. (359) reported transient accumulation of PAH ("overshoot") under anaerobic conditions when kidney slices, depleted of Na^+, were transferred to a medium containing Na^+ and PAH. The effect appeared to be specific for Na^+, and the authors suggested that under aerobic conditions active transport of PAH may be driven by the difference in electrochemical potential of Na^+ across the basolateral membrane, created by the Na^+-K^+-ATPase. In previous studies of vesicles, Na^+-dependent transport of PAH against a concentration gradient could not be demonstrated in basolateral membrane preparations from the rat (39) and dog (252) kidney. However, in our own studies with a basolateral membrane preparation of rabbit kidney, we obtained unambiguous evidence of an "overshoot" of PAH in the presence of a Na^+-gradient directed from the extravesicular to intravesicular space (see fig. 3). Rather than being due to species variations it seems more probable that this different result is due to alterations in the preparative procedure, including a treatment with bivalent cations to obtain a less leaky preparation. In addition, we observed a Na^+-gradient dependent "overshoot" of PAH uptake in Na^+-K^+-depleted slices of rat kidney under anaerobic conditions, thus suggesting that Na^+ cotransport is not a phenomenon specific for the rabbit kidney. Under aerobic conditions PAH accumulation by slices of rabbit kidney cortex at various Na^+-K^+ concentrations was correlated with the Na^+-K^+-ATPase activity of the preparations (309). An involvement of Na^+-K^+-ATPase in the active transport of PAH is corroborated by electrophysiological measurements in the isolated newt kidney; this shows an inverse relation between intracellular PAH uptake and the electrochemical potential of Na^+ across the basolateral membrane (250). Taking all these studies together, cotransport of Na^+ and PAH seems to be reasonably well established. However, it should be noted that PAH fluxes across the renal membranes are dramatically increased by O_2-metabolism after thorough Na^+-depletion, which suggests a role of metabolism to increase PAH permeation by a Na^+-independent mechanism (307).

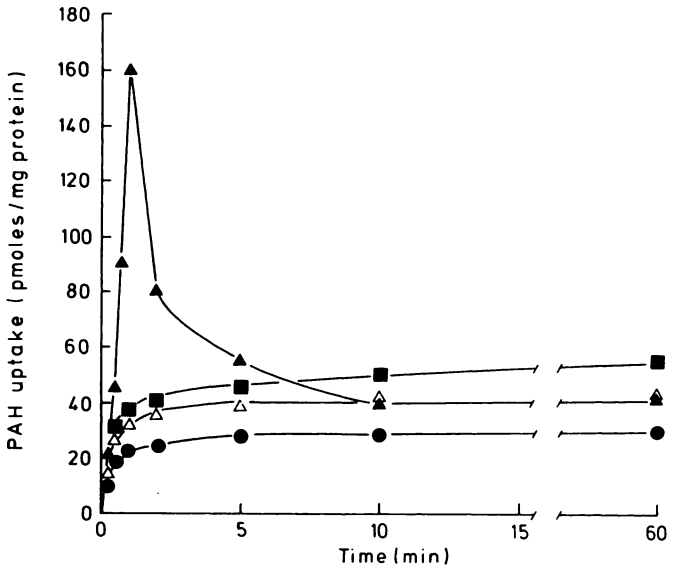

FIG. 3. Uptake of PAH by basolateral and luminal membrane vesicles suspended in different media. The vesicles were prepared by differential centrifugation after homogenization and pretreatment with a medium containing 2.5 mM Mg^{++}, 2.5 mM Ca^{++}, 300 mM sucrose, and 25 mM Hepes/Tris buffer (pH 7.0). The final step in the preparation of basolateral membranes included density gradient fractionation with Percoll, and the vesicles were stored in a 300 mM sucrose and 25 mM Hepes/Tris buffer (pH 7.0). The media used for the uptake experiments in addition to 25 mM Hepes/Tris (pH 7.0) and 0.075 mM PAH with ^{14}C-labeled PAH contained 150 mM NaCl (▲——▲), 150 mM KCl (△——△) and 150 mM NaCl + 1 mM probenecid (●——●) in the case of basolateral membrane vesicle experiments. (■——■) denotes uptake of PAH by luminal membrane vesicles in the presence of 150 mM NaCl gradient. For further details, see Sheikh and Møller (428).

5. Effect of Ouabain on PAH Transport. Burg and Orloff (74) observed that PAH accumulation by rabbit kidney slices is inhibited by strophantidin and that this effect can be counteracted by a rise in the medium concentration of K^+. Subsequently the inhibitory effect of ouabain on PAH accumulation in vitro was confirmed by many investigators (104, 184, 308, 358, 448). The inhibitory effect of the glycoside is correlated with the inhibition of the Na^+-K^+-ATPase in the preparation (309, 448). Low concentrations of vanadate, which act as an inhibitor of Na^+-K^+-ATPase, also lead to inhibition of PAH accumulation in rabbit kidney slices without other discernible effects on the renal metabolism (424). In microperfusion experiments, Woodhall et al. (528) observed a drastic decrease by 10^{-4}M ouabain on the transcellular transport of PAH by isolated fragments of proximal tubules of rabbit kidney. However, ouabain did not affect PAH transport in the golden hamster in vivo, despite an effect of the glycoside on glucose and phosphate reabsorption (488). Accordingly, the transport of PAH quantitatively may show a different cation dependency than other transport functions of the tubule.

6. Energization of PAH Transport. The primary role of Na^+ in active reabsorption of many compounds in the proximal tubule fluid, e.g. of D-glucose and amino acids, has been established in the past decade (for recent reviews, see 328, 402, 403, 486). Reabsorption of solute occurs by cotransport with Na^+ across the luminal membrane and is energized by the electrochemical Na^+-gradient. As discussed above Na^+-K^+-ATPase activity also appears to be involved in transcellular transport of PAH in the rabbit kidney, but whether the Na^+ gradient alone is sufficient to account for the observed intracellular accumulation of PAH is not certain at the present time. If we suppose that the transport is electroneutral, i.e. that PAH and Na^+ are transported in a 1:1 ratio (250,

428), the maximal intracellular accumulation ratio, PAH_i/PAH_o, that can be achieved by the thermodynamic energy of the Na^+ gradient is given by

$$PAH_i^-/PAH_o^- = Na_o^+/Na_i^+ \quad (1)$$

In the kidney slice system the intracellular Na^+ concentration of the proximal tubule cells may be around 30 to 40 mM (358, 448), corresponding to a maximal accumulation of PAH around 4 to 5. But even the attainment of this maximal value implies no appreciable saturation of the PAH-carrier complex with Na^+, i.e. that the dissociation constant for binding of Na^+ is much higher than the Na^+ concentrations in the extracellular and intracellular medium in contact with the membrane. At least in the rabbit kidney Na^+ seems, in fact, to have a rather high affinity for the PAH-carrier complex, since PAH accumulation in kidney slices from this animal is only affected to a modest extent by decreasing the medium concentration of Na^+ to 30 mM (184, 185, 309). It therefore appears unlikely that for electroneutral transport the Na^+ gradient is capable of sustaining experimentally measured values of PAH accumulation. This could mean either that Na^+-dependent PAH accumulation requires cotransport of more than one Na^+*, or that metabolism is also involved in other ways in the energization process. Evidence that metabolism drives or potentiates part of PAH transport by a mechanism, unrelated to Na^+-K^+-ATPase activity, is that low concentrations of CN^-, 2,4-dinitrophenol and F^- cause a reduction of PAH accumulation without any detectable effect on intracellular Na^+-K^+ concentrations (307). Furthermore, metabolic stimulation of PAH accumulation by acetate, lactate, and pyruvate is not accompanied by any detectable effect on Na^+ transport in the slices (420). An intriguing possibility is that PAH accumulation may be supported by countertransport with anions being formed by metabolic processes used to sustain Na^+-K^+-ATPase activity (39, 420, 485). Direct evidence that PAH can be accumulated by anion-exchange is lacking, but SITS (4-acetamido-4′-isothiocyano-2,2′-disulfonic stilbene), which is a strong inhibitor of the erythrocyte Cl^-/HCO_3^- exchange system, has been reported to inhibit PAH accumulation by kidney slices (225).

7. Transport of PAH Across the Luminal Membrane. While there is general agreement concerning the presence of an active transport step for PAH at the basolateral membrane, opinions differ on the mode of transfer of PAH across the luminal membrane. The high intracellular concentration of PAH, in conjunction with the potential difference across the luminal membrane (cell interior negative) (180), obviates the need for active transfer of PAH from cell to tubule lumen. Wedeen and Vyas (499) reported an enhancement of intracellular accumulation of PAH after addition of phlorizin to the medium used for incubation of the kidney slices. Phlorizin, which blocks transport of sugars across the luminal membrane, impeded the transport of PAH from cell to tubular lumen. Accordingly, a phlorizin-sensitive step for PAH transport across the luminal membrane was suggested.

The presence of probenecid-sensitive transport of PAH across the luminal membranes was suggested by Foulkes (172) in experiments in which the urinary excretion pattern of PAH and inulin in the rabbit was compared by collection of timed samples from a ureteral catheter after bolus injection of the two compounds into the renal artery. When the experiments were performed after administration of a relatively small dose of probenecid, just sufficient to almost block the tubular secretion of PAH, the urinary excretion of PAH was delayed relative to that of inulin; this suggests that glomerularly filtered anion entered renal cells to a significant extent during transit through the nephrons. However, coincidence between transit times could be obtained after pretreatment with a higher dose of probenecid, which suggests that the drug under these conditions also inhibited carrier-mediated transport across the luminal membranes.

Several studies have been performed on luminal membrane vesicles, but these have so far not given a clear-cut result. Berner and Kinne (39) found that uptake of PAH by luminal membrane vesicles of rat kidney occurred slowly and was unaffected by probenecid, in contrast to PAH uptake by a basolateral membrane fraction. Eveloff et al. (148) studied PAH uptake in the flounder kidney and found that in this species a distinct accumulation step is involved in the transfer from cell to lumen (166). PAH uptake by the luminal membranes was inhibited in particular by 2,4-dinitrophenol and SITS but not to any significant extent by probenecid. On the other hand Kippen et al. (258) reported that a preparation of luminal membranes from rabbit kidney took up PAH by a process that was partially inhibited by probenecid and other organic anions. Kinsella et al. (252) found that uptake of PAH by luminal and basolateral vesicles from dog kidney exhibited quite similar characteristics with respect to PAH uptake. For both preparations a saturable, probenecid-sensitive component in the uptake curve could be detected. The luminal membrane vesicles were characterized by a higher K_m for transport (3.9 mM) than the basolateral membranes (0.5 mM).

In none of the above studies was an "overshoot" of PAH uptake observed in the presence of a Na^+-gradient. In an interesting new development Blomstedt and Aronson (48) reported that a probenecid sensitive "overshoot" could be demonstrated in Na^+-free medium in the presence of a transmembranal H^+ gradient (medium pH < intravesicular pH). This was attributed to a PAH^-/OH^- exchange or a PAH^--H^+-cotransport mechanism. In the presence of a simultaneously occurring Na^+-H^+ exchange mechanism at the luminal membrane such a process may

* In the case of fluorescein Bresler and Nikiforov (67) have suggested cotransport with 2 Na^+, but this is not considered likely to apply to PAH transport (67, 250, 428).

be anticipated to lead to net transport of PAH from tubular fluid to cell, i.e. to reabsorption of PAH. Clear evidence for reabsorption of PAH and diodrast is found in the Necturus kidney (253, 257, 471). Reabsorption in this species contributes to intracellular accumulation of PAH, and is more susceptible to inhibition by octanoate than the secretory process. In other species a similar reabsorptive process for PAH has not been demonstrated with certainty, but a small fraction of PAH is reabsorbed after microinjection of this compound into the lumen of proximal tubules in rats (9, 446). Furthermore, the appearance of PAH in urine in these experiments is delayed relative to that of inulin; this suggests a relatively high permeability of the luminal membrane to PAH in accordance with the kinetic model of Tune et al. (478). Cho and Cafruny (89) obtained evidence of transcellular transfer of PAH during retrograde ureteral infusion of a solution containing PAH, glucose, inulin, and mannitol. Subsequent collections of urine samples derived from the proximal tubule were identified by having a deficit of PAH and glucose, relative to that of inulin. In addition, appearance of PAH in renal venous plasma was inhibited by administration of probenecid. Although apparent reabsorption of PAH by the rat nephron is slight, Weiner (501) has pointed out that microinjection experiments may underestimate the true extent of this process, since subsequent re-entry of reabsorbed PAH into the urine is likely to occur.

8. *Effect of Metabolites on PAH Accumulation.* Cross and Taggart (102) originally demonstrated that the accumulation of PAH is enhanced by acetate, pyruvate, and lactate, while short-chain fatty acids like hexanoate and octanoate, and dicarboxylic acids such as α-ketoglutarate, fumarate, and succinate inhibit the uptake of PAH when added to the medium in millimolar concentrations. The inhibitory effect of short-chain fatty acids was later attributed to partial conversion to acylglycines which are substrates of the organic anion transport system (407). Furthermore, the effect of acetate was assumed to be the result of deinhibition of PAH accumulation as a result of reduced formation of endogenous acylglycines (407). An impressive support for the acylglycine hypothesis was the demonstration that the stimulatory effect of acetate on PAH accumulation was observed only in kidneys from species that are capable of synthetizing acylglycines. However, it is doubtful whether acylglycine formation can quantitatively account for the decrease in PAH accumulation in the absence of acetate (501). It is more likely that the stimulatory effect of acetate is a consequence of stimulation of renal metabolism. Studies from our laboratory (418, 431) and by Kippen and Klinenberg (259) have shown that the diverse effect of metabolites on organic anion transport is not as sharp as was originally supposed to be the case. Thus fatty acids, fumarate, succinate, malate, and citrate at low medium concentrations produce at least as much stimulation of PAH accumulation as does acetate. The reduction of PAH accumulation at high-medium concentrations of these metabolites probably is due to a direct inhibitory effect on the transport system. A possibility currently under investigation concerning the metabolite stimulation of PAH transport is that it is caused by an increased formation of dicarboxylate compounds which enhance PAH transport by a Na^+-independent, anion-exchange mechanism (420).

B. Urate

1. *Species Differences.* Extensive data are available on the excretion of urate in various species, because of the great interest that pertains to the metabolism of urate as a pathogenic factor of gout, but the renal handling of urate is characterized by an unprecedented complexity as compared to that of other organic anions. This situation in part reflects marked species variations both in regard to the extent of tubular secretion and reabsorption, and in the response of these processes to various drugs. A detailed account of this subject is given in the book edited by Kelley and Weiner (246). For the purpose of this review, which primarily deals with the relation of transport of urate to that of other organic anions, as a framework for discussion we shall adopt the following broad division of urate excretion in various species into three groups:

A. SPECIES WITH A STRONG SECRETORY COMPONENT, WITH OR WITHOUT SIMULTANEOUS REABSORPTION OF URATE. This group includes uricotelic organisms such as birds and many reptilian species, where no definitive evidence for an independent reabsorptive process has been provided (109). The group also includes several mammalian species such as the rabbit (23, 319, 362), Dalmatian dog (176, 526), guinea pig (327), pig (393, 437), and goat (325). Due to the presence of uricase in these mammalian species, the plasma level of urate is low and under these conditions the excreted amount most often is close to, or below, that filtered by the glomeruli, i.e. there is net reabsorption of urate. Net secretion usually becomes evident during infusion of urate, but there are large variations within individuals of the same species. In some cases demonstration of secretion has required special maneuvers such as the establishment of stop-flow during infusion of urate and mannitol (23, 327). Organic anions competing for tubular transport such as probenecid, PAH, and salicylate, when effective only give rise to a decrease in urate excretion in these species.

B. STRONG ABSORBERS OF URATE. Examples are man, chimpanzee, and the Cebus monkey. Urate excretion in these species is around 5% to 20% of glomerular filtration (152, 200, 439). Urate excretion, especially in chimpanzee and man, is subject to modification by a variety of organic anions like probenecid, carinamide, salicylate, pyrazinoate, phenylbutazones, diuretics, and x-ray contrast agents (146, 389, 438). Drug effects may be biphasic, e.g. small amounts of salicylate depress urate excretion, while

high doses have a uricosuric effect (538). Pyrazinoate has a dramatic urate retaining effect both in man (535) and chimpanzee (158) and has been shown to be uricosuric at high dosage in the latter species. Net secretion has occurred spontaneously in some humans with hypouricemia (249, 363, 436) and during combined infusion of urate, mannitol, and sulfinpyrazone (201). In the chimpanzee net secretion of urate may be elicited by administration of mersalyl (154). The Cebus monkey in comparison to chimpanzee and man exhibits a blunted response to drugs (159). The prevailing opinion, based on several clearance studies involving drug administration, is that secretory fluxes in the three species are high, despite the fact that the major part of urate normally is reabsorbed from the tubule (158, 160).

C. MAMMALIAN SPECIES WITH INTERMEDIARY CHARACTERISTICS. Examples are rats, mongrel dogs, and cats (325, 325a, 389). In these species urate normally is excreted below the glomerular level, also during administration of urate. Evidence of net secretion in the mongrel dog has been reported by the combined infusion of urate and an osmotic diuretic (287, 533). Net secretion of urate during infusion of this compound to rats (388) and dogs (391) has been observed by micropuncture in some of the punctured proximal tubules during free-flow, although urinary excretion did not exceed the glomerular level. These species are generally characterized by the lack of an appreciable effect of drugs under free-flow conditions, but the direction of change, whenever measurable, tends to be the same as in man (325). The lack of an appreciable drug response in many cases may be the result of an almost equally inhibitory effect on tubular reabsorption and secretion as evidenced by clearly demonstrable drug inhibition of secretory flux of urate, e.g. by pyrazinoate (282) and probenecid (514) in the rat, and by PAH, salicylate, and chlorthiazide in the mongrel dog (542).

2. Site of Urate Transport. It is generally accepted that the proximal tubule is the site of carrier-mediated secretion and reabsorption of urate [see reviews (389, 502)]. Despite earlier reports that suggested secretion at a distal site (112, 293, 532), this is probably not the case (389). Reabsorption of urate in the loop of Henle (190) has been reported and also may occur in the distal part of the nephron (281), but only to a limited extent (389, 502).

3. Steps of Tubular Secretion of Urate. The methodology developed by Tune et al. (478) for examination of tubular secretion in isolated tubules has been used by Dantzler (106) to study the transtubular transfer of urate in a reptilian species (Thamnophis). Urate is accumulated by the renal cells as a consequence of active transport across the basolateral membrane of the proximal tubule. The concentration of urate in the tubule perfusate is lower than in the tubule cells, so that transfer across the luminal membrane occurs downhill, along a concentration gradient. In comparison with PAH, urate tubular secretion is inefficient, characterized by a lower T_{max} and a higher K_m (107). The amount of urate added to the tubule perfusate is markedly dependent on perfusion rate. In contrast to PAH, low perfusion rates reduce the amount of urate collected in the perfusate. In order to account quantitatively for efflux data obtained by addition of urate to the perfusate, Dantzler (106, 108, 109) suggested that urate may undergo reabsorption from tubule fluid through the paracellular pathway. However, the existence of a significant role of back-diffusion through the tight junction may be questionable, since PAH, with a similar molecular weight and charge, apparently is not reabsorbed by that pathway (section IV D). Weiner (502) has pointed out that an active reabsorption may exist across the luminal membrane of the snake tubule, in analogy with active reabsorption of PAH in Necturus (253). This may account for the high permeability constant for efflux and for the observation that intracellular accumulation of urate is reduced in nonperfused tubules (106), since under these conditions a contribution from tubule fluid to cellular accumulation does not exist, or is at least severely curtailed. Alternatively, the existence of an intricate system regulating the active transport step across the basolateral membrane presumably has to be postulated (109).

Similar studies have recently been published on tubular transport of urate in the rabbit. Chonko (91) found a secretory flux both in the pars convoluta and pars recta, which was inhibited by probenecid, ouabain, and incubation in the cold. However, only in pars recta fragments did the urate concentration in the perfusate appreciably exceed that of the bath. In studies on microdissected, nonperfused tubules of rabbit proximal tubules Schäli and Roch-Ramel (409) found that the S_2-fragments exhibited the highest accumulation of urate (approx. 20 times). This is in contrast to snake tubules, in which urate secretion occurs evenly over the whole of the proximal tubule (106). The assignment of the S_2-fragment by these authors as the site of predominant urate secretion in the rabbit was also indicated by free-flow micropuncture experiments, where net reabsorption was observed in the first part of the proximal tubule (391). However, Shimomura et al. (433) subsequently reported that microperfused S_1, S_2, and S_3 segments have about the same capacity for urate secretion at high concentrations of urate in the bath. A sigmoid curve described the relation between probenecid-sensitive transfer of urate to the tubule fluid and bath concentration. The authors suggested that the carrier for urate secretion has allosteric features that result in inefficient transport at low concentrations of urate in the peritubular fluid.

4. Relation between Tubular Secretion of Urate and PAH. It was found in early studies (performed between 1959 and 1968) that many drugs such as probenecid, sulfinpyrazone, phenylbutazone, and salicylate are uricosuric in man but depress the tubular secretion of both urate and PAH in the chicken (25, 330). In the rabbit PAH and diodrast inhibited the tubular secretion of urate in a strictly competitive manner (320). Urate also inhibited the excretion of PAH, but high plasma concen-

trations were required, which indicated low affinity for the transport system. Furthermore, urate secretion was inhibited by the same compounds that caused a decrease of PAH excretion (320, 321). It was concluded from these studies that urate and PAH probably share a common secretory pathway in the chicken and rabbit kidney. At about the same time Zins and Weiner (542) showed that the secretory flux of urate, as studied by injection of urate and inulin into the renal artery of mongrel dogs during stop-flow, was inhibited by PAH, chlorothiazide, and salicylate. Administration of probenecid or PAH also was shown to result in urate retention in the Dalmatian dog (248, 534) and guinea pig (327).

On the basis of the evidence presented above, one might have anticipated that urate secretion universally occurs by the hippurate system, but a more complex picture has emerged from studies performed during the last decade which, together with other evidence (section III A), points to a differentiation of the organic anion secretion system. Dantzler's studies in the reptile kidney unequivocally show the presence of different secretory systems for urate and PAH in this species. The most decisive arguments are based on the absence of competition between the two compounds (105, 107) and a different segmental pattern of transport: Urate is secreted over the whole proximal tubule, while PAH secretion predominantly is localized to the distal region of the proximal tubule (106, 107). In man and chimpanzee, pyrazinoate has a distinct urate retaining effect in contrast to that for chicken (25, 330) and rabbit (409). In the rat, microinjection experiments into peritubular capillaries have indicated that the secretory flux of urate in this species is inhibited by pyrazinoate (1.6 to 3.2 mM). On the other hand, the presence of a high concentration of PAH (1.5 to 6.4 mM) did not affect the secretory flux of urate (282). Studies involving injection into the renal artery and subsequent collections from the renal vein also indicate dissimilar secretion of PAH and urate in the rat (171), but a similar pattern in the dog and rabbit (241, 334). Direct demonstration of an inhibitory effect of pyrazinoate on the secretory flux of urate in the Cebus kidney has also been reported (392, 392a). PAH has little, if any, retaining effect on urate excretion in man (52) and chimpanzee (160), while the secretion of pyrazinoate is readily inhibited by PAH (157, 312).

The observations made in rat, man, and chimpanzee can only reasonably be interpreted on the assumption of two different secretory systems for PAH and urate, respectively, in these species (502). Similar results and conclusions have been drawn concerning the urate retaining effect of *m*-hydroxybenzoate in the Cebus monkey (312). A basic difference between urate and PAH secretion in the chimpanzee is also indicated by strong competition of mersalyl with PAH and diodrast for secretion, while net secretion of urate may be obtained by administration of the diuretic at a similar dosage (152).

On the other hand urate and PAH secretion have many features in common in species like the rabbit, chicken, pig, and Dalmatian dog. But, even in these species it is questionable whether transport occurs by the same system. Although in the rabbit this reservation is mainly based on a perfusion study by Shimomura et al. (433), it is also on the basis of some differences in sensitivity to Na^+-K^+ (see below) and on the substrate induction during development (section III C).

Many aspects of the relation between secretion of organic anions thus remain unexplored. It is doubtful whether pyrazinoate and *m*-hydroxybenzoate, despite their inhibitory effect, are actually substrates of the urate secretory system. In effect the ready inhibitability of the secretion of these compounds by PAH (157, 312) suggests that they are secreted by the hippurate system. Furthermore, probenecid inhibits the secretory flux of urate in the rat (514) and reptile (106), where the secretory systems for PAH and urate are definitely different. The secretory flux of urate in the mongrel and Dalmatian dog is inhibited by both PAH (394, 532, 542) and pyrazinoate (334, 394, 532). These results suggest that discrete transport systems for urate and PAH have overlapping substrate specificity in many species.

5. Intracellular Accumulation of Urate. Studies on the uptake of urate by cortex slices have shown that the compound is accumulated intracellularly in species such as rabbit, guinea pig, chicken (357), and snake (104) with a strong secretory component in the tubular handling of urate. On the other hand, the steady-state uptake of urate in kidney slices from dog, man, rat, and chimpanzee (357) was below that of the incubation medium. Accumulation of urate is inhibited by anoxia (260, 357, 426). The following observations suggest that intracellular accumulation of urate in the rabbit is due to active uptake across the basolateral membrane as part of tubular secretion of this compound: a) Urate accumulation in separated tubules of the rabbit is competitively inhibited by PAH (409, 426); b) Urate inhibits PAH accumulation in the S_2-segment of the rabbit, while pyrazinoate has little effect on either urate or PAH accumulation (409); and c) the accumulation of both urate and PAH in the rabbit is affected in the same way (stimulation and/or inhibition) by addition of various metabolites to the incubation medium (259).

Sheikh and Møller (426) found slice-medium ratios below unity in rabbit kidney slices at low-medium concentrations of urate. This conforms to the fact that secretion is observed in vivo only after a rise of the peritubular concentration of urate in this species (319, 362). Slice-medium ratios below unity have been attributed to extrusion of urate across the basolateral membrane as part of the reabsorptive process for urate (322, 426).

In contrast to other authors, Jones and Despopoulos (242) were unable to detect intracellular accumulation of urate at any level of medium concentration, although the compound inhibited the accumulation of PAH (119). Whether this result could have arisen from the use of rabbits with a high reabsorption of urate (23) is unknown.

6. Na^+-K^+-Dependence of Tubular Secretion of Urate. In the perfused snake tubule preparation Dantzler (104) found that the presence of K^+ in the bath was necessary to obtain appreciable transport of urate, while replacement of NaCl with choline chloride had no effect. This was in contrast to PAH transport, which was reduced by removal of either Na^+ or K^+ from the bath. Dantzler concludes that in the snake, urate is specifically stimulated by K^+. On the other hand Chonko (91) found that 10^{-4} M ouabain almost completely inhibited the secretory flux of urate in the rabbit. Urate accumulation in slices of both rabbit (35) and chicken kidney (104) is abolished by ouabain, which indicates that Na^+-K^+-ATPase is involved in urate transport as in the case of PAH (section II A). However, a specific effect of K^+ on urate secretion may also exist in other species than the snake. This urate accumulation, in contrast to that of PAH, is not reduced by medium concentrations of K^+ higher than 40 mM in the rabbit (35) and chicken (104). Berndt and Beechwood (35) have suggested that the specific stimulatory effect of K^+ could be due to a stimulation of tubular reabsorption of urate, since in clearance experiments in the rabbit they found that ouabain inhibited tubular reabsorption of urate. However, further studies are required to define the nature of the effect of high concentrations of K^+ on urate transport.

7. Tubular Reabsorption of Urate. Several observations suggest that urate is reabsorbed by a carrier-mediated process:

A. INHIBITION OF NET REABSORPTION OF URATE BY DRUGS. An increase in urate excretion to, or above, the level of glomerular filtration has been observed after administration of mersalyl to the chimpanzee (154), sulfinpyrazone in conjunction with osmotic diuresis in man (201), and by tienilic acid in the dog (292). These findings are consonant with the view that the predominant part of urate reabsorption occurs by a carrier-mediated mechanism.

B. REABSORPTION OF URATE AGAINST AN ELECTROCHEMICAL GRADIENT. This has been reported to occur in the rat in microperfusion experiments with no net fluid absorption (515), and in the Cebus monkey after administration of pyrazinoate (392). U/P values lower than unity have been reported to occur in man after administration of pyrazinamide (542) and after intravenous infusion of lactate to man (459) and rabbit (316). It should be noted that the demonstration of U/P values below unity requires that the concentration of urate at the end of the proximal tubule is much lower than that of plasma. Otherwise the urinary concentration of urate would rise above the plasma concentration in the final urine because of abstraction of water from the distal part of the nephron, which is relatively impermeable to urate. If we further assume that the electric potential difference across the proximal tubule of man and chimpanzee is close to zero as in other species (54), we can conclude that urate reabsorption has the characteristics of an active transport process, i.e. it may occur against an electrochemical gradient.

C. THE EXISTENCE OF AN APPARENT T_{max} FOR URATE REABSORPTION IN SOME SPECIES. Infusion of urate leads to a marked increase in the urinary excretion of urate in man (29, 533) and Cebus monkey (155). Analysis of clearance data is consistent with an apparent T_{max} for urate reabsorption. However, prior administration of pyrazinamide leads to almost complete disappearance of urate from tubule fluid, even during infusion of urate (151, 202). Therefore, the apparent T_{max} in the absence of pyrazinoate probably reflects a balance between tubular reabsorption and secretion. Such a balance could arise if, above a certain level of urate in tubule fluid, there is a one-for-one exchange of urate by reabsorption and secretion. A coupling between reabsorption and secretion could occur if, for example, one step in the transtubular transfer mechanisms is common. However, urate reabsorption and secretion are characterized by both qualitative and quantitative differences in the susceptibility to inhibition by drugs (section IV D). Therefore, the hypothesis probably requires the existence of transport steps at both the luminal and basolateral membrane, and that only one of these is common for both reabsorption and secretion (see below).

An important factor governing the extent of urate reabsorption is the state of the extracellular volume. During extracellular volume expansion in man by saline infusion, urate excretion is augmented (83, 131, 301), while the converse is the case after extracellular volume contraction following administration of furosemide and ethacrynic acid (458, 464, 511). There is at present insufficient evidence to decide whether there is a specific dependence of urate reabsorption on Na^+. Zins and Weiner (542) found a decrease of both urate and Na^+ reabsorption during infusion of CN^- into the renal artery of dogs. Beechwood et al. (23) reported that urate reabsorption was inhibited by administration of ouabain to the rabbit. On the other hand the presence of a Na^+-gradient does not result in an "overshoot" uptake of urate by luminal vesicles from the rabbit kidney (55a, 258).

But here, the possibility that the rabbit is probably a species with a poorly developed reabsorptive system (319) must be taken into consideration. Another possibility is that reabsorption occurs by an urate-OH^- exchange or, alternatively, an urate-H^+ cotransport mechanism (48, 245). This view is derived from the demonstration of an "overshoot" of urate uptake by a luminal membrane preparation of dog kidney in the presence of a H^+-gradient (pH of medium larger than pH of intravesicular fluid).

In order to clarify the nature of urate reabsorption microperfusion experiments are also needed. However, some complications arise in such studies from the simultaneous presence of reabsorption and secretion. Thus attempts to estimate unidirectional fluxes have led to conflicting results such as a higher apparent secretory

than reabsorptive flux in the rat (286, 388), despite evidence of net reabsorption of urate (394).

8. Site of Uricosuric Effect. A large body of experimental material suggests that the inhibitory effect of drugs on urate reabsorption in man is related to the concentration in tubule fluid rather than to the plasma level. This was first noted by Yü and Gutman (538) who found that salicylate provoked uricosuria more readily during alkalosis than during acidosis, where a larger fraction of the drug is reabsorbed from the tubule fluid. The dependence of uricosuria on the presence of the drug at an intratubular location has also been demonstrated in primate species by concomitant administration of a second substrate that inhibits tubular secretion of the uricosuric drug. PAH diminishes the uricosuric effect of 2-nitroprobenecid by inhibition of tubular secretion and thereby decreases the availability of the drug at its site of action (160). This site could be located either at the luminal membrane or, alternatively, at the cellular aspect of the basolateral membrane. On the other hand, pyrazinoate had a definite urate-retaining effect in the presence of probenecid (160, 540); this suggests that the site of action is on the extracellular aspect of the basolateral membrane.

9. Mechanism of Tubular Transport of Urate. The model of urate transport shown in figure 4 is speculative, but incorporates features of tubular transport of urate as discussed in the preceding paragraphs, relating to species with appreciable reabsorption. Urate is considered to be reabsorbed from the tubular fluid to the intracellular space by an anion exchange system, located at the luminal membrane. This step represents active transport, powered by Na^+-H^+-exchange (180) or, more directly, by cellular metabolism. It is subject to inhibition by various uricosuric drugs such as probenecid and salicylate in man, chimpanzee, and Cebus monkey. Urate is removed from the cells to the peritubular fluid down an electrochemical gradient across the basolateral membrane. Furthermore, there is an active transport of urate in the opposite direction across the basolateral membrane which can be inhibited by pyrazinoate, and probably also by other organic anions, in man, chimpanzee, Cebus monkey, and rat. Since the cell content has a negative electrical potential (186), this will constitute a driving force for a lower concentration of urate in the intracellular than in the peritubular fluid. However, the actual intracellular concentration of urate will be determined by the balance of influx and efflux. Under conditions where the transport of urate from the peritubular fluid to cell is high, this may lead to intracellular accumulation and net transport of urate from the cell to luminal fluid across the luminal membrane. The model may also account for the apparent T_{max} for reabsorption observed in man (29, 533) and Cebus monkey (155) in the absence of pyrazinoate as being the result of an increased urate-urate exchange across the luminal membrane, contingent upon a rise in the intracellular urate concentration as the result of the elevated plasma concentration of urate.

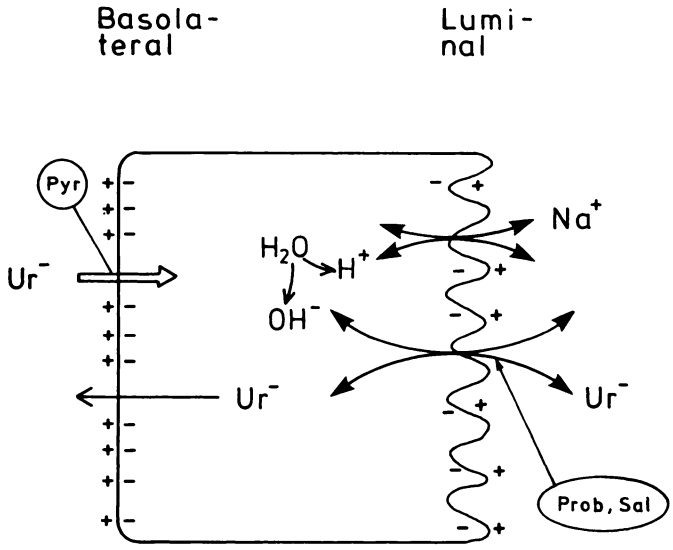

FIG. 4. Model of bidirectional transport of urate. A pyrazinoate sensitive system is situated at the basolateral membrane. Uptake at the luminal membrane occurs in exchange with OH^- by a salicylate and probenecid sensitive system. For further explanation, see text.

C. Phenolsulfophthalein Dyes

Secretion of anionic dyes offers the opportunity of microscopic visualization of the transport process. Phenolsulfophthalein (PSP) dyes, among which phenol red is the parent compound, have been particularly well examined. Many of the present concepts concerning the organic anion transport are derived from studies on the tubular secretion of PSP dyes which are therefore reviewed below.

1. Steps of Transtubular Transport. Undifferentiated tubules of the aglomerular kidney appear capable of secreting phenol red into their lumen against a large concentration gradient from cell to lumen. This was demonstrated in cysts of tubular epithelium formed in tissue culture from the mesonephros of the chicken (81, 86) and in preparations of separated tubules from many fish (teleost) species (255). In particular, flounder kidneys have been used for transport studies. Separated tubules can be obtained simply by teasing of the kidney tissue (164). The tubules form elongated cysts with closed ends. Due to the osmotic effect of secreted dye the tubules have an open lumen and are therefore well suited for microscopic visualization of the dye uptake process. It was found that rapidly transported dyes such as phenol red and chlorphenol red do not give rise to a visible staining of the renal cells when the tubules were suspended in a balanced electrolyte medium (169, 170), but when Ca^{++} was omitted, dye was seen to accumulate inside the cells (369). If K^+ was omitted from the medium, the dyes were not accumulated at all (224, 369). Thus, dye secretion in the flounder kidney appears to be characterized by two active steps, viz. K^+-dependent uptake

at the peritubular membrane and Ca^{++}-dependent uptake of dye at the luminal membrane. Autoradiographic studies have indicated appreciable intracellular accumulation of phenol red, despite the absence of visible staining of the tubular cells (255). A direct demonstration of carrier-mediated transport at both the luminal and basolateral side of the cell has been reported to occur for PAH in purified vesicle preparations (148) (see section II A). The specificity of the dependence of dye secretion on Ca^{++} and K^+ is doubtful, since there is morphological evidence of changes in the membrane structure in the absence of these cations (70). In particular, it appears probable that lack of Ca^{++} leads to diffusion of dye from the lumen to the medium between the cells, due to an opening of tight junctional complexes (255).

In contrast to the flounder kidney an accumulation step solely across the basolateral membrane has been demonstrated in the rabbit (167, 427) and rat (396, 427, 447) kidney. In the rabbit kidney, uptake of dye is most prominent in the pars recta, in accordance with the prowess of this segment for tubular secretion of other organic anions. The same is true in the rat kidney, but in this case the preferential dye uptake by pars recta is insensitive to administration of probenecid, in contrast to the rabbit kidney (427). Other histological observations suggest that tubular reabsorption contributes to intracellular accumulation of phenol red or chlorphenol red by the pars recta in this species (447).

2. Relation between Tubular Secretion of Various PSP Dyes. In addition to the flounder kidney, transport of various PSP dyes has been studied in the chicken (454), dog (251, 296), and rabbit (226, 418, 419, 183). Despite differences in the steps involved in tubular transfer, the tubular excretion shows similar features in all species studied, and the results may be summarized as follows:

a. PSP dyes show a great disparity in the extent to which they are secreted, but a lower T_{max} is normally accompanied by a higher affinity for the transport system. Thus both T_{max} and K_m decrease in the same order: phenol red > bromphenol blue > bromcresol green > bromthymol blue. The last mentioned dye is only transported to a very small extent (183, 454).

b. In agreement with the existence of a common transport system, dyes with a low K_m have a strongly inhibitory effect on the tubular secretion of the more readily transported dyes (166). Inhibitors of organic anion transport such as probenecid, 2,4-dinitrophenol, octanoate, or succinate affect the secretion of phenol red to a higher extent than that of the substituted PSP dyes (419).

c. PSP dyes are bound to renal tissue constituents by a passive process (149, 348, 418, 419). The affinity and extent of binding of substituted dyes, in particular that of bromthymol blue, is higher than for phenol red (418). Forster (166) at an early stage noted that many of the substituted dyes accumulated intracellularly and were only very sluggishly transferred to the lumen of the flounder tubule, a phenomenon which was referred to as intracellular "trapping" of dye.

Comparisons between the distribution coefficient of PSP dyes between octanol:water and binding by liposomes and serum albumin indicate that hydrophobic interactions presumably play a major role for the strong binding of some of the substituted dyes (419). As in the case of probenecid and homologues (section II D), a pronounced degree of hydrophobic interaction seems to result in decreased transport rates and a higher affinity for transport.

Binding studies on homogenate fractions of cortex tissue have shown that the dyes are bound both to the various membrane fractions and to cytosolic proteins (149, 418, 419). Binding can be partially inhibited by probenecid and 2,4-dinitrophenol, but not by PAH, octanoate, and succinate (419). Eveloff et al. (149), in addition to low affinity binding, described the presence of high affinity sites for phenol red (2–4 μmole/g of protein with a dissociation constant of $\simeq 10^{-4}$ M), localized to the microsomal fraction. The possibility that this might represent binding to the carrier was suggested. However, the rather high binding capacity as compared to the expected number of carriers and the lack of inhibition by PAH seriously question the validity of the proposal. Although probenecid and 2,4-dinitrophenol do inhibit phenol red binding, high concentrations are required, and it appears probable that the effect may be ascribed to a nonspecific competition for nonpolar sites in the membrane lipid and proteins. Probenecid and homologues are also bound by kidney tissue with similar characteristics as those of PSP dyes (430–432). Concerning the nature of the cytosolic binding proteins, little is known, except that "ligandin," a GSH S-transferase, interacts strongly with organic anions (205, 262). However, the amount of ligandin is too low to account for more than a small fraction of the observed binding.

3. Clearance Experiments. The existence of a T_{max} at high dye concentrations for tubular excretions of phenol red in various species has previously been assumed (441), but newer studies show a "self-depression" of tubular excretion of chlorphenol red (504) and phenol red (183), consistent with the simultaneous presence of tubular reabsorption of dye. Net reabsorption of phenol red is observed after blocking of tubular secretion with probenecid (183). Under these conditions reabsorption of phenol red is dependent on urinary pH in such a way as to suggest reabsorption of the acid (univalent anionic) form in preference to the basic (bivalent anionic) form of the indicator dye (183). Reabsorption by non-ionic diffusion seems a less likely possibility. Firstly, the pK_a of the uncharged dye is low, i.e. can only be detected in strong, concentrated acids. Secondly, phenol red at pH 5 to 8 does not readily leak out of liposomes, in contradistinction to probenecid (183).

In contrast to PAH, a major fraction of phenol red at low dye concentrations is not extracted from renal plasma

during a single passage through the kidney (183, 335, 443). The low extractability may be accounted for in part by plasma protein binding (335) and in part by tubular reabsorption (183). Phenol red excretion is easily inhibited by other organic anions such as diodrast and PAH, while phenol red has little effect on the tubular excretion of these two compounds (441). In this respect, there is a curious difference in the apparent affinity of phenol red and PAH in vivo and in vitro, since inhibitors of organic anion transport consistently reduce PAH accumulation in kidney slices to a larger extent than that of phenol red (418). The reason for this difference between in vivo and in vitro results has not been determined.

D. Probenecid and Other Homologues of Sulfamylbenzoate

Probenecid (p-di-n-propylsulfamylbenzoic acid) occupies a central place in the field of organic anion transport because of its inhibitory potency. The compound is characterized by rather slow elimination under ordinary (nonalkalotic) conditions so that a single dose suffices to produce a sustained effect. Eventually, most of the drug is excreted in glucuronidated form together with various oxidation products and monopropylsulfamylbenzoic acid (113, 314, 353). Alcohols and carboxylic acid derivatives predominate among the oxidation products and are the result of limited oxidation of the propyl side chains (314).

The low rate of urinary excretion led Beyer (40) to suggest that the compound is a "refractory" substrate, i.e. it binds to but is not transported by the organic anion transport system. However, Weiner et al. (509) in clearance experiments found that probenecid excretion is drastically augmented after alkalinization of the urine; this results in net secretion of the compound. Under these conditions, the renal excretion of probenecid could be inhibited by administration of PAH, thus demonstrating that the drug actually is secreted by the organic anion transport system. The low excretion under nonalkalotic conditions is attributed to non-ionic diffusion (501, 509). In accordance with this view, probenecid is observed to diffuse quickly through the lipid of multilayered liposomes (183).

The first studies of the uptake of probenecid by kidney slices indicated that the drug was accumulated both under a 100% O_2 (62) and a 100% N_2 atmosphere (31, 32). No significant difference between the aerobic and anaerobic accumulation of probenecid was observed, which suggests lack of active uptake of the compound. Unlike transport of PAH in vitro, uptake and run-out of probenecid from kidney slices were unchanged by many metabolites and metabolic inhibitors (33). However, a subsequent study with ^{14}C-labeled probenecid, enabling low medium concentrations of the compound to be used, revealed that probenecid is accumulated in kidney slices both by an active transport process and by passive binding (431). The active component was sensitive in the same way as PAH to the presence of metabolites, metabolic inhibitors, and other secreted organic anions in the medium. These findings establish probenecid as a true substrate of the renal organic anion transport system in accordance with the clearance results obtained by Weiner et al. (509). Presumably, the higher concentrations and nonspecificity of the chemical procedure in the first studies precluded the detection of the active component of uptake. Anaerobic uptake of probenecid is due to binding to renal membranes and proteins in the cytosol (431). The binding is not affected by PAH, but can be inhibited in part by octanoate and phenol red which also exhibit unspecific binding to kidney tissue constituents (section II E).

1. Homologues of Probenecid. The hydrophobic properties of probenecid are largely attributable to the presence of the two *n*-propyl side chains. A systematic variation of the hydrocarbon chain length affords an opportunity to test the effect of hydrophobicity on the renal handling of the compounds. It was noted by Weiner et al. (509) that an inverse relationship exists between hydrocarbon chain length and renal clearance, in accordance with the idea that non-ionic diffusion is important for the tubular reabsorption of these compounds. In addition, the uptake characteristics of these compounds by kidney slices have been examined (432). The compounds studied were the dimethyl $(CH_3)_2$-R, diethyl $(C_2H_5)_2$-R, and dibutyl $(C_4H_9)_2$-R analogues of probenecid, where R = sulfamylbenzoic acid. In addition to probenecid, $(CH_3)_2$-R and $(C_2H_5)_2$-R were transported by the organic anion transport system as evidenced by aerobic accumulation in kidney slices with the following characteristics: a) stimulation by acetate as a metabolic substrate; b) biphasic response by addition of fumarate and octanoate to the incubation medium; and c) inhibition by PAH. The affinity of the compounds for transport increased with the length of the hydrocarbon substituent. Platts and Mudge (357) found the same inhibitory potency of the same compounds toward accumulation of PAH and urate in rabbit kidney slices. Uptake of $(C_4H_9)_2$-R is characterized by a high degree of anaerobic binding to the tissue slices with little extra uptake under aerobic conditions. The behavior of this compound approaches that of a "refractory" substrate. Efflux rates were similar for all the compounds, i.e. non-ionic diffusion did not seem to play any important role for dissipation of concentration gradients between tissue and medium in the kidney slice system. This somewhat surprising observation raises the question of whether the major part of non-ionic diffusion occurs in the distal part of the nephron, i.e. in other cells than those involved in active uptake (see also section II E).

Similar studies on the uptake of monosubstituted sulfamylbenzoic acid derivatives (ranging from CH_3-R to C_5H_{11}-R) showed that both affinity and transport increased as a function of the length of the hydrocarbon substituent (430). The partitioning of the compounds

between a nonpolar and aqueous phase indicated that they were less hydrophobic than probenecid, and only C_4H_9-R and C_5H_{11}-R were bound to tissue constituents to a noticeable degree. Thus, a moderate degree of hydrophobicity seems to enhance transport, while affinity for the transport system is increased by an increase of hydrocarbon chain length. A quantitative analysis indicated strict competition of the monosubstituted compounds with PAH for the transport system, while probenecid accumulation was reduced to a somewhat smaller extent than expected. The basis for this difference may either be the presence of various subsystems for organic anion transport (section III A) for which probenecid is a common substrate, while the monoalkylated sulfamylbenzoates are not; or it may reflect a different mode of interaction of probenecid with the same transport system, caused by increased hydrophobic interactions with the carrier (430).

E. Salicylate, Metabolites, and m-Hydroxybenzoate

In ordinary therapeutical conditions, salicylate (*o*-hydroxybenzoate) is extensively reabsorbed by the renal tubules. Clearance values corresponding to, or higher than, glomerular filtration rate, are obtained at low plasma concentrations of the drug and during alkalosis and a high diuresis (103, 203, 406, 508, 519). This is consistent with a role of both tubular secretion and non-ionic diffusion in the renal handling of salicylate (508). Metabolites of salicylate, arising from glucuronidation of the phenolic and carboxylic group, and salicylurate (*o*-hydroxybenzoylglycine) have a stronger tendency for net secretion, their clearance values approaching renal plasma flow (406). However, net reabsorption has been observed to occur for salicylurate at high plasma concentrations (504). Participation of the compounds in organic anion secretion is documented by a depressing effect of probenecid and PAH, also under conditions of net reabsorption (203, 406, 505). In a recent micropuncture study, net secretion of salicylate was demonstrated in the early proximal tubule of the rat, while reabsorption occurred in the distal tubule (390). Reabsorption was also demonstrated in the late proximal tubule, particularly at low plasma salicylate concentrations and in the absence of bicarbonate in the systemic infusion fluid. However, the augmentation of salicylate concentration in the beginning of the distal tubule fluid during bicarbonate infusion, compared to that during mannitol infusion, was less than anticipated for reabsorption by non-ionic diffusion in the late proximal tubule (390). On the other hand, the authors considered that the extent of reabsorption of salicylate by the distal tubule was consistent with non-ionic diffusion. As an alternative to non-ionic diffusion, it is possible that there is a carrier-mediated system for salicylate in the proximal tubules coexisting with tubular secretion. This possibility is of interest in relation to results obtained on the renal handling of the isomer *m*-hydroxybenzoate. As mentioned in section II B, this compound shows great inhibitory affinity toward urate secretion in the Cebus monkey. Studies on the renal handling of *m*-hydroxybenzoate in the dog (311) and Cebus monkey (312) indicate similar characteristics of the excretion of *m*-hydroxybenzoate and salicylate (504) as a function of the plasma concentration of ultra-filterable compounds—a tubular secretory component of limited capacity that is saturated at low concentrations, and a larger reabsorptive component. However, in contrast to salicylate, excretion of *m*-hydroxybenzoate is not influenced by changes in urinary pH and diuresis (311). According to stop-flow experiments, reabsorption of *m*-hydroxybenzoate occurs in the proximal tubule. Definite evidence for active reabsorption in the dog is provided by the observation of U/P ratios of 0.3. Furthermore, there is some evidence for saturation of *m*-hydroxybenzoate reabsorption at high plasma concentrations (311).

In conclusion, only carrier-mediated reabsorption seems to exist for *m*-hydroxybenzoate, whereas salicylate may be reabsorbed both by non-ionic diffusion and carrier-mediated transport. Lack of non-ionic diffusion of *m*-hydroxybenzoate may be due to a lower lipid solubility than that of salicylate (the partition coefficient in chloroform:water for *m*-hydroxybenzoate is 0.01, as compared to 2.9 for salicylate, but on the other hand, the pK_a for *m*-hydroxybenzoate (4.1) is more favorable for non-ionic diffusion than that of salicylate (pK_a 3.0), data from Weiner et al. (504).

F. Phenylbutazone, Sulfinpyrazone, and Analogues

These compounds are derived from a diphenyl-3,5-pyrazolidinedione with substituents at the positions indicated in figure 5. The acidic nature of the compounds is derived from proton dissociation by the heterocyclic ring. According to the nature of the substituents, the pK_a values may differ over a wide range, from 2 to 5.5 (197). Phenylbutazone and sulfinpyrazone are examples of a representative compound with a relatively high (4.5), and low (2.8) pK_a value, respectively (197). There is an inverse relation between pK_a and rate of renal excretion in man and dog (79, 198). All compounds are lipophilic and

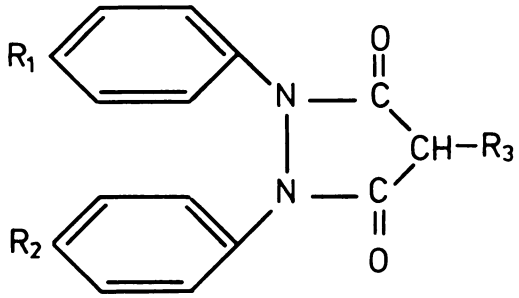

FIG. 5. General formula for phenylbutazone and analogues. Typically R_1 and R_2 represent small substituents like −H, −OH, −Cl, −F and $-SO_2CH_3$, and R_3 a larger substituent. For phenylbutazone $R_1 = R_2 = H$ and $R_3 = -(CH_2)_3CH_3$; for sulfinpyrazone $R_1 = R_2 = H$ and $R_3 = -(CH_2)_2SO_2C_6H_5$.

interact extensively with plasma proteins (binding ≃ 98% in humans) which hamper studies on renal excretion. Thus, phenylbutazone was excreted below the detection limit of a spectrophotometric assay in man (198). However, comparative studies in the dog on the excretion of phenylbutazone, sulfinpyrazone, and other analogues were possible (198), presumably as a consequence of less protein binding (≃ 92%). Firm evidence was obtained for tubular secretion of all the compounds examined during alkalosis. Without infusion of bicarbonate or during stop-flow the excretion of phenylbutazone dropped below glomerular filtration rate, presumably because of dominant non-ionic diffusion. By contrast the excretion of sulfinpyrazone and another acidic compound was almost unaffected by changes in urinary pH, and a proximal peak was evident during stop-flow. The higher level of excretion of the acidic compounds was attributed in part to a higher rate of tubular secretion, and in part to the absence of appreciable non-ionic diffusion.

The uricosuric effect of phenylbutazone, sulfinpyrazone and other analogues is correlated with the rate of urinary excretion, i.e. there is an inverse relation between uricosuria and pK_a (197).* Despopoulos et al. (127) were unable to find a similar correlation with regard to inhibitory potency on PAH transport in vitro. The only consistent structural-functional relationship was that hydroxylation decreased inhibitory affinity. In this connection it is of interest that hydroxylation also significantly reduces the hydrophobicity (198), a factor which is an important determinant of inhibitory affinity of the drugs for the transport system (section III B). In discordance with Despopoulos (120), other studies indicate a stronger inhibitory effect of the more acidic compound sulfinpyrazone than of phenylbutazone on PAH (357) and urate (260) accumulation in rabbit kidney slices.

Phenylbutazone inhibits tubular secretion of PAH in vivo (536), but after intravenous administration of comparable doses the inhibitory effect of phenylbutazone is less pronounced and more transient (127) compared to that of probenecid (44). On the other hand phenylbutazone is at least as effective as probenecid in the inhibition of PAH or urate accumulation in rabbit kidney slices (260, 357). Despopoulos (120) suggested that differences between in vivo and in vitro results might be dependent on biotransformation of phenylbutazone in vivo. However, in acute experiments this seems to be an unlikely possibility, since metabolism of this group of compounds is a slow process, involving hydroxylation at specific carbon atoms producing other active metabolites, before glucuronidation (132). Instead the modest effect of phenylbutazone on tubular secretion of PAH in vivo probably may be related to the extensive plasma protein binding of the drug.

* In contrast, the anti-inflammatory effect of some members of this group of compounds is more specific and unrelated to uricosuria (130).

G. Pyrazinoate and 5-Hydroxypyrazinoate

Interest in the renal excretion of pyrazinoate stems from the fact that it is a rather specific inhibitor of urate secretion in man and many other species. A urate-retaining effect is also observed after administration of pyrazinamide, an antituberculous drug (537), but the active compound is pyrazinoate, formed by deamidation of pyrazinamide in vivo (507). After administration of pyrazinoate to dog, man, and Cebus monkey recovery in the urine was complete after 24 h and comprised 5-hydroxypyrazinoate, pyrazinoate, and sometimes minor amounts of a second metabolite, tentatively identified as pyrazinoylglycine (507). Conversion of pyrazinoate to 5-hydroxypyrazinoate presumably is catalyzed by xanthine dehydrogenase, since the formation of 5-hydroxypyrazinoate can be inhibited by prior administration of allopurinol (507). These observations have been confirmed by other authors (6, 143).

Clearance experiments by Weiner and Tinker (507) revealed that: 1) In the dog, pyrazinoate is reabsorbed by an active, carrier-mediated process; the excretion of pyrazinoate is inhibited to some extent by PAH, which suggests concomitant tubular secretion. 2) Net secretion of pyrazinoate at low plasma concentrations can be demonstrated in the Cebus monkey, but reabsorption is the major mode of tubular handling at high plasma concentrations. 3) Net secretion of 5-hydroxypyrazinoate was found consistently in various species (man, dog, Cebus).

Among other species evidence for secretion of pyrazinoate has been obtained in the chimpanzee (157) and guinea pig (327). There is, however, no correlation between net secretion of pyrazinoate and the inhibitory effect on urate secretion in various species (389). Secretion of pyrazinoate in the chimpanzee is readily inhibited by PAH and other organic anions transported by the hippurate system (157), whereas it has not been possible to demonstrate inhibition of PAH secretion by pyrazinoate (52, 157). These observations suggest that secretion of pyrazinoate may occur by low affinity interaction with the hippurate system, in addition to the marked inhibitory effect of the compound on the urate secretory system. In any event, the data are not compatible with the presence of only one secretory system for all three organic anions (502); cf. discussion in section IIB. The excretion of 5-hydroxypyrazinoate is also inhibited by PAH, but this compound does not appear to have a urate-retaining effect (507). Pyrazinoate inhibited the excretion of xanthine, but not of hypoxanthine, in a xanthinuric subject (6). This finding may suggest a common transport system for secretion of urate and xanthine in man.

The reduction of urate excretion in man following administration of a suitable dose of pyrazinamide has been proposed as a measure of tubular secretion of urate (202). The validity of the pyrazinamide suppression test has been seriously questioned (158, 219), since it presupposes that secreted urate is not reabsorbed, i.e. that urate

secretion is located distal to the reabsorptive process. However, as discussed in section II B, tubular secretion of urate occurs concomitantly with urate reabsorption in the proximal tubule. Furthermore, Fanelli and Weiner (157) have shown that in the chimpanzee large doses of pyrazinoate induce uricosuria. An inhibitory effect of pyrazinoate on tubular reabsorption of urate, also at a dosage level giving rise to a minimum of urate excretion, cannot be excluded.

H. Diuretics

1. Diuretic Effect. Diuretics that have been shown to be secreted are assembled in groups II, III, and V of table 1. They constitute a heterogeneous group of compounds, including furosemide, bumetanide, and piretanide (group II); ethacrynic acid, indanone (MK 196), ozolinone, and mersalyl (group III); and the thiazides (group V). Most of the compounds are "loop" diuretics, i.e. the diuretic effect is primarily dependent on inhibition of NaCl transport in the ascending loop of Henle (71). However, thiazide diuretics primarily inhibit fluid absorption in the distal tubule (99, 283). At high concentrations the diuretics may also exert some inhibitory effect on proximal tubule reabsorption which in part may be accounted for by an inhibitory effect on carbonic anhydrase (376, 411). The chronic and acute administration of diuretics is often attended by a fall in glomerular filtration rate which may obscure an effect on proximal tubule reabsorption, unless glomerular filtration can be restored to normal level by salt and water replacement (78, 93). Since the diuretics are also vasoactive agents they may be anticipated to affect the magnitude and distribution of blood flow within the kidney. After administration of furosemide and ethacrynic acid, an increased renal blood flow has been observed, which has been suggested to be involved in the diuretic effect (349, 460). There are observations that do not favor this view—thus, bumetanide, a potent diuretic closely related to furosemide, does not increase renal blood flow (177). The levorotatory enantiomer of ozolinone has a diuretic effect, in contrast to the dextrorotatory form, yet both isomers increase blood flow to the same extent (195).

2. Secretion of Diuretics. A common feature of the diuretics considered here is that they are strongly bound to plasma proteins. This puts a limitation on glomerular filtration so that in many cases tubular secretion becomes an important determinant of urinary excretion. Most studies are available on the renal handling of furosemide. After intravenous injection in man furosemide is eliminated with a half-life of less than 1 h (7, 182, 289, 496), and most of the compound is excreted in unaltered form in the urine within 24 h (3, 24, 95). Tubular secretion of furosemide was first demonstrated by a micropuncture study in the rat kidney (115). The renal clearance of furosemide in man in therapeutic dosage (uncorrected for protein binding) is at the same level, or somewhat higher, than inulin clearance (221, 222). Since plasma protein binding in man is about 90% to 95%, clearance values of this magnitude indicate appreciable tubular secretion of the compound. In the absence of protein in the perfusate furosemide clearance was equal to that of PAH in the perfused rat kidney preparation (59), showing that the diuretic has a high affinity for the secretory system and that tubular reabsorption is not significant. However, the secretory rate of furosemide was reduced by the inclusion of albumin in the perfusate (59). Accordingly plasma protein binding seems to exert a depressing effect on furosemide secretion, presumably because of less availability of unbound furosemide for secretion. Autoradiographic studies have shown that furosemide first is taken up by the proximal tubular cells and subsequently is delivered to the distal part of the nephron where the diuretic effect is exerted (118, 472). Pretreatment with probenecid alters the diuretic response of furosemide. This has been shown in the dog (229) and the cat (177) where infusion of the diuretic in an intrarenal artery caused an ipsilateral increase in the Na^+ excretion that was abolished by probenecid. A luminal effect of furosemide, dependent on tubular secretion, has also been demonstrated in the hen by the use of the Sperber technique (338).

A somewhat more complicated picture has emerged from studies in humans: pretreatment with probenecid resulted in a small decrease (222), no effect (221), or an increase (61) in the overall diuretic effect of furosemide (diuresis for 6 to 24 h after a single intravenous injection). It should be noted that the doses of probenecid used in these studies gave only partial inhibition of tubular secretion of furosemide. Under these conditions the inhibition of tubular secretion of furosemide may therefore be compensated for by a higher glomerular filtration rate of furosemide as the result of an increased plasma concentration. In agreement with this view, the diuretic effect correlated well with the urinary excretion of furosemide in the studies of Homeida et al. (221) and Odlind and Beerman (341). The explanation of cases with a larger total diuretic effect of furosemide after probenecid administration may be that probenecid also inhibits extrarenal elimination of furosemide (221, 496). In conclusion, the pharmacological effect of furosemide presumably is determined by the amount that arrives at Henle's loop with the tubular fluid as the result of both secretion in the proximal tubule and glomerular filtration.

Among the thiazide group of diuretics an inverse relation exists between secretory potential and hydrophobicity (42, 135), similar to what is observed for PSP dyes and probenecid analogues (section II, C and D). A dependence of diuretic effect on tubular secretion, suggesting a luminal site of action, has been demonstrated for ethacrynic acid (196, 339), piretanide (339), and tienilic acid (340, 445). Bumetanide is an interesting exception. Since the diuretic effect is not influenced by a dosage of probenecid that blocks the diuretic effect of furosemide in the cat (177), possibly the diuretic gains access to the luminal membrane from the blood side because of a high degree of hydrophobicity (95). In a study in the hen by

Odlind (338), with the modified Sperber technique, the diuretic was secreted at a low rate and only caused a slight ipsilateral excess of water and electrolyte excretion as well. In the case of chlorothiazide, Odlind (337) observed the opposite phenomenon—the ipsilateral diuretic effect was pronounced and was not affected by inhibition of tubular secretion. These observations suggest a basolateral site of action of the diuretic.

It is of interest that both stereoisomers of ozolinone are secreted, but only the *l*-form exerts a diuretic effect on Henle's loop in micropuncture experiments, with no interference by the enantiomer (195). On the other hand *d*-ozolinone is capable of inhibiting proximal tubular secretion of the *l*-form (195). This is an example of the less specific structural requirements for affinity of organic anions for organic anion secretion than exists in the case of most other transport systems (section III B).

3. Effect on Urate Excretion. The chronic administration of most diuretics to man results in hyperuricemia as a result of renal retention of urate. In acute experiments with intravenous administration of furosemide and ethacrynic acid, a marked decrease in urate excretion was not observed before appreciable salt and water loss had occurred (458). Furthermore, the fall in urate excretion could be prevented by replacement of salt and water losses, both in acute experiments (458) and during chronic administration of diuretics (464, 510, 511). Therefore, the effect is probably indirect and caused by a contraction of extracellular volume. Other conditions such as diabetes insipidus and restricted salt and water intake, giving rise to extracellular volume contraction, are also accompanied by urate retention (219). Steele (457) has suggested that urate secretion may be inhibited as the result of a diminished blood flow to the kidney, resulting in less availability of urate for transport across the basolateral membrane. However, the decrease in glomerular filtration may also contribute to the reduction of urate excretion. At any rate urate retention is unlikely to result from any direct effect of the diuretic on transtubular transport of urate. Immediately after the administration of furosemide and ethacrynic acid there is a slight increase of urate excretion in man and chimpanzee (117, 150), which suggests some inhibition of urate reabsorption. With some diuretics a distinct and prevailing uricosuric effect is observed. Thus, after administration of mersalyl to the chimpanzee, Fanelli et al. (154) demonstrated a startling increase in urate excretion to net secretory levels. In man a somewhat less pronounced increase in urate excretion was observed after the acute administration of this diuretic. Indanone (MK-196) also causes a pronounced and protracted increase in the level of urate excretion in the chimpanzee (150, 154a) and rat (512). In human studies interest has focused on tienilic acid (179). The chronic administration of this drug leads to pronounced hypouricemia in man (288, 291, 375). The uricosuric effect of the drug is unrelated to the diuretic effect that is localized to the ascending limb of Henle's loop (463, 513). In species with a predominant urate secretion tienilic acid inhibits urate excretion (292, 294), presumably as the result of competition for tubular secretion (445).

I. Antibiotics

Despite the fact that penicillin was one of the first chemotherapeutics for which tubular secretion by the organic anion system was demonstrated (45, 373, 374), there is a paucity of data on the excretion of this and other antibiotics. The early studies on penicillin showed high clearance values, which indicated almost complete extraction from the renal plasma by passage through the kidney. On the other hand Barza et al. (21) found only partial extraction of benzylpenicillin (less than 40%) in the dog. Since these authors used much higher plasma concentrations this observation would be consistent with an approach to saturation of the secretory mechanism under these conditions. If so, the data of Barza et al. (21) indicate that the T_{max} is quite low, corresponding to perhaps 5 to 10 μmol/min in the dog. Evidence that benzylpenicillin has a high affinity for transport is provided by a strongly inhibitory effect on the transport of other organic anions (46). Despite a general lack of clearance studies there is presumptive evidence that semisynthetic penicillins are secreted by the human kidney, insofar as urinary excretion is inhibited by simultaneous administration of probenecid as is the case with ancillin (401) and ampicillin (142). Barza et al. (21) found that nafcillin is extracted from the renal plasma in the dog kidney, but in contrast to benzylpenicillin the liver was the major route of excretion of this antibiotic.

Cephalosporins constitute another group of antibiotics that are transported by the kidney. Like the penicillins, they are derivatives of penicillic acid. Most interest has focused on the renal excretion of cephaloridine, because of the nephrotoxic potential of this compound (360). Active transport apparently plays a dominant role for development of necrosis of proximal tubular cells as evidenced by the following findings: 1) correlation between ability of the antibiotic to accumulate intracellularly and nephrotoxicity in various species (477); 2) a protective effect of probenecid and PAH on development of necrosis (482); and 3) correlation between cephaloridine toxicity and development of the organic anion transport system in the newborn rabbit kidney (525). The rabbit kidney is particularly sensitive to cephaloridine and accumulates the compound to a higher extent than PAH (476, 479). This contrasts with the urinary excretion of the compound, which is very close to that of inulin clearance (88, 517). On the basis of several experimental observations, Tune et al. (480) conclude that transtubular transport of the antibiotic is minimal or nonexistent, i.e. excretion at the glomerular level cannot be accounted for by concomitant secretion and reabsorption of equal magnitude by the renal tubules. The luminal membrane is considered to constitute the permeability barrier to transtubular transport of cephaloridine; this results in

high intracellular levels as the consequence of active transport across the basolateral membrane and impeded outflow from the proximal cells. By contrast, distinct secretion is demonstrable in the case of cephalothin (290) which, like most other cephalosporins, does not have the same nephrotoxic potential as cephaloridine. However, newer observations suggest that factors other than the intracellular concentration also play a role for the relative nephrotoxic potential of various cephalosporins (481).

The majority of antibiotics, which are not derivatives of penicillic acid, are not acidic compounds and are thus not expected to be substrates of the organic anion transport system. However, among the streptomycins an acidic antibiotic is known, novobiocin ($pK_a \simeq 4.2$), which has been shown to be an effective inhibitor of organic anion transport (181, 337).

J. Conjugated Compounds

The role of the organic anion system for the elimination of "detoxification" compounds was brought into focus by the classical studies of Sperber (451–453) on the biosynthesis and renal secretion of various conjugates by the renal portal system of the hen. The most important types of compounds are conjugation products with glycine, glucuronic acid, and sulfate. Both liver and kidney possess enzyme systems for conjugation of susceptible precursors (272, 497) arising from the diet, metabolic products of intestinal bacteria, or after administration of drugs. Among the glycine conjugated compounds hippurate is the substance that is excreted in large amounts in normal urine, and it is predominantly formed from benzoate or precursors of benzoate in the diet, such as chinoic acid (1, 49). Hippurate constituted 15% to 28% of the total amount of excreted aryl acids in normal and uremic subjects fed on an ordinary diet (84). Other aromatic glycine conjugates of substituted benzoate have been identified in the urine of uremic patients (213, 280). Inborn errors of amino acid metabolism often give rise to the excretion of glycine conjugates of aliphatic carboxylates, e.g. isovalerylglycine, tiglylglycine, and 3-methylcrotonylglycine (20). Glucuronidation is involved in the elimination of steroid hormones (28, 134) and many drugs, e.g. salicylate (406), phenylbutazone (132, 133), probenecid (113, 314, 353), and paracetamol (136). Glucuronidation of aromatic anions occurs concomitantly with glycine conjugation, and in differing proportions in various species (272, 372). For steroid hormones sulfation of hydroxyl groups also occurs in addition to glucuronidation (28, 134). Furthermore, sulfation is involved in the elimination of catecholamine (383, 452), morphine (310, 498), phenol (452), and indoxyl (56).

An increased concentration of arylglycines and sulfates has been detected in uremic sera (56, 187, 361). This may account for the decreased renal extraction of PAH in patients with diseased kidneys and for a pronounced inhibitory effect of uremic sera on PAH accumulation in kidney slices (58, 228, 344). In contrast to PAH the tubular excretion of tetraethylammonium is not affected in dogs rendered azotemic by bilateral uretero-venous anastomosis (313). The contributory role of an accumulation of organic anions to the manifestation of the uremic disease has been discussed by Grantham et al. (189, 361).

Conjugation converts inert precursors to substrates for transport in the case of benzoate and aliphatic carboxylates (section III B), simple phenolic compounds (452), and catecholamines (371, 378). However, Sperber (452) was unable to detect secretion of pregnandioleglucuronide. Brown et al. (68) found net secretion for estriolglucuronide in pregnant women, but net reabsorption of estrone- and estradiol-17β-glucuronide. For the latter two compounds, excretion was reduced by administration of probenecid. There is little concrete evidence on the role of conjugation for transport affinity, except for electroneutral precursors. In these cases conjugation results in the formation of anionic compounds, which is a prerequisite for transport (section III B). It is probable that conjugation also may affect the renal excretion of hormones, for example, by causing less reabsorption by nonionic diffusion and less binding to plasma proteins as the result of an increased water solubility.

Another factor to be taken into account is that, in addition to liver, substantial conjugation takes place in the kidney (271, 497), and this may have consequences for the renal handling. Thus, it has been shown that probenecid, as expected, inhibits tubular excretion of administered morphine ethereal sulfate (310, 498) and catecholsulfate (383). However, the renal excretion of these compounds is not affected by probenecid after administration of the precursors morphine (310) and catechol (370, 371). The plausible interpretation of these data is that the conjugates in these experiments were synthetized by the kidney, and that the conjugates were transferred directly from there to the luminal fluid by a process that was not inhibited by probenecid.

III. General Aspects of Organic Anion Transport

A. Subsystems of Organic Anion Transport

The renal secretion of organic anions comprises a wide range of chemical structures and is characterized by a pronounced degree of species variability as seen in the case of urate (section II B). Evidence accumulated during the past decade indicates that organic anion secretion probably is differentiated into subsystems with a narrower substrate specificity. It is instructive to start by comparing organic anion secretion in kidney and liver. Both organs are capable of excreting the same organic anion substrates, e.g. PAH (123), PSP dyes (125, 527) bromsulfalein (527), and x-ray contrast agents. Yet there are quantitative differences in affinity for transport, since PAH and phenol red are excreted preferentially by the

kidney, while bromsulfalein exhibits higher affinity for the liver. In general large and hydrophobic substrates seem to be taken up by the liver. For example, nafcillin (6-(2-ethoxy-1-naphthamido)penicillate) is predominantly extracted by the liver, in contrast to benzylpenicillin which is almost exclusively excreted by the kidney (21).

Among x-ray agents, iodipamide is excreted preferentially by the liver, whereas diodrast and hippuran are predominantly secreted by the kidney (14). Nevertheless, Bárány (14) found a component of high affinity, active uptake of ^{125}I-iodipamide by rabbit kidney slices, which could be completely inhibited by addition of high concentrations of unlabelled iodipamide. Uptake of radioactive iodipamide could also be inhibited by PAH, but a substantial fraction of accumulated iodipamide was insensitive to PAH, even at very high medium concentrations (10 to 30 mM). From these observations it was concluded that iodipamide accumulation occurs by at least two systems, one of these being identical with the hippurate system and the other similar to the liver system.

Bárány (14) also reported the presence of transport of iodipamide by both the hippurate and liver-like system in uvea and plexus choroideus of rabbit, monkey, and guinea pig. Iodipamide uptake by dog kidney was much lower than in the rabbit kidney. Interestingly, this result is correlated with in vivo studies by Berndt and Mudge (38) who found evidence of tubular secretion of iodipamide in clearance experiments in the rabbit, but not in the dog.

Bárány (16) found that other x-ray agents with an affinity for liver were also capable of inhibiting PAH-insensitive uptake of iodipamide in rabbit kidney slices, whereas renographic contrast agents like diodrast and hippuran were not. By testing the effect of many organic anions, he came to the conclusion that, in general, organic anions with a large molecular weight (>200) inhibited PAH insensitive iodipamide uptake. However, some caution in the interpretation is required, because uptake was not measured under anaerobic conditions to evaluate the possible binding of organic anions by tissue constituents. It is striking that many of the compounds that were reported to affect PAH insensitive iodipamide uptake are bound by renal membranes and proteins, e.g. probenecid, phenylbutazone, sulfinpyrazone, and substituted PSP dyes.

There are other probable instances of differentiation of the organic anion transport system. We have already considered in detail the relation between urate and PAH secretion (section II B). Transport of bile acids has been attributed to a separate transport system with characteristics intermediary between that of PAH and iodipamide (17). Secretion of prostaglandins is inhibitable by other organic anions, but larger concentrations of PAH are required than anticipated for competitive inhibition by the same system (46). Another case of specialization of organic anion transport is the renal secretion of oxalate. Weinman et al. (512) reported, on the basis of micropuncture experiments, that oxalate is secreted in the early part of the proximal tubule by a probenecid insensitive mechanism. However, in other studies PAH, probenecid, and urate had an inhibitory effect when oxalate excretion was studied over the whole of the proximal tubule (191, 268). To account for these differences Knight et al. (268) assume that oxalate is excreted by two different systems, one that is insensitive to probenecid and located in the convoluted part of the proximal tubules, and the other occurring by the hippurate system in pars recta of the proximal tubule. D-Malate is secreted by the proximal tubules of the dog by a probenecid insensitive process (492). Although citric acid cycle intermediates (including the physiologically occurring L-malate) are not secreted by the kidney, there is evidence from vesicle studies for uptake across the basolateral membrane by a separate, probenecid insensitive mechanism (420).

The impression gained on the basis of the available evidence is that even for organic anions whose secretion is inhibited by probenecid, various subsystems exist with a spectrum of overlapping specificities, i.e. binding specificity of an organic anion to a particular subsystem in most cases is probably not absolute. Hence, the possibility exists that the same substrate may be transported by more than one subsystem. On the other hand, binding affinity apparently is not always equated with good transport properties; e.g. pyrazinoate has high affinity for urate secretory system in some species, but apparently is itself secreted by the hippurate system (507). Furthermore, PAH seems to inhibit thiosulfate secretion without itself being readily transported by that system (490). In future studies there is clearly a need for more work to define the properties of subsystems.

B. Specificity of Renal Organic Anion Secretion

1. Chemical Structures Involved in Transport. In an early analysis from 1965 Despopoulos (122) summarized the available information on the substrate specificity of the renal organic anion system. The approach used was to define common features in the diverse group of substrates that could account for their transport properties. The proposals were often of a speculative nature and to some extent provocative, as also admitted by the author. Unfortunately, the challenge has not been taken up in any serious way by other research workers.

The most extensive analysis was performed on the hippurate class of compounds, mainly based on previous work done in particular by Knoefel and Huang who elucidated the transport properties of a large number of structural analogues of hippurate substituted benzoates (233, 270, 271, 273, 279). Despopoulos came to the conclusion that the general structure shown in figure 6 describes the minimal requirements for transport. In —NH— the hydrogen atom does not contribute, since

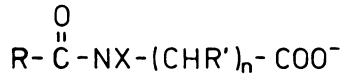

FIG. 6. General formula of transported hippurates. The carbonyl and carboxylate groups are those critical for transport. R may be an aliphatic or aromatic group, R' is an aliphatic group, n = 1–5. Taken from Despopoulos (122).

substitution with —CH_3 results in a compound which is transported as well. An effect of nitrogen can also be excluded, since analogues in which it is lacking are substrates. The radical R in the formula may represent an aromatic or an aliphatic group; n may take on all values between 1 (as in hippurates) and 5, for example p-aminobenzoyl-δ-aminovalerate is a substrate. The findings provide rather strong evidence for a critical role of the carbonyl and carboxylate group in the secretion of hippurates. Accordingly, it was suggested that interaction with the carrier requires a three-point attachment that involves hydrogen bond formation of the carrier with the carbonyl and the two oxygens of the carboxylate group in a reinforced ionic bond.

The difficulty inherent in this approach is that, although it may give a satisfactory description of the transport properties within a given class of compounds, it cannot be used to predict the behavior of other types of compounds that are also substrates of the system. Despopoulos (122) attempted to overcome this difficulty by postulating that a set of equivalent groups exist that may replace the hippurate side chain in the interaction with the carrier. For instance the ionized sulfonate group of phenol red, and the ionized sulfonamide (—SO_2—NH^-—) in certain sulfonamides that are secreted (126), has the potential to form two hydrogen bonds and one ionic bond with about the same spatial configuration as the hippurate side chain in the folded form. However, in order to account for the secretion of some of the compounds considered, Despopoulos (122) felt it necessary to postulate that the carrier might also interact with the extended form of the hippurate side chain.

Quite apart from the speculative nature of these considerations, it seems certain that important factors are lacking from the analysis. For instance salicylate (o-hydroxybenzoate), m-hydroxybenzoate, and phenylacetate are substrates, yet they do not conform to the three-point attachment theory (501). Although the compounds could be excreted by a different subsystem than PAH, competition phenomena indicate that in all likelihood they are capable of interacting with the same carrier molecules. Accordingly, a wider base must be found to encompass all the features of secreted compounds. It seems certain that the phenyl group of these substrates must be involved in the interaction indicating that there exists a binding site for an aromatic ring. This view is also in keeping with the presumed function of the system in the elimination of substrates that are not easily degradable by metabolic processes. The interaction may be hydrophobic (of unspecific nature) or there may be a binding site which sterically fits the aromatic ring and perhaps provides favorable interactions with the π electrons. The latter possibility is the more probable, since: a) Some aromatic heterocycles, with no or less pronounced hydrophobic characteristics, may be accepted as substrates, e.g. pyrazinoate, diodrast, and urate. b) Long-chain fatty acids presumably are not substrates, although at high concentrations they interfere with organic anion transport (102, 305).

Schachter et al. (407) attributed the inhibition by fatty acids to formation of acylglycines. However, it is doubtful whether acylglycine formation quantitatively can account for the inhibition of PAH transport (501). The simplest hypothesis is that fatty acids directly inhibit PAH transport. This view is supported by the demonstration of an inhibitory effect on PAH transport under anaerobic conditions (429). Barac-Nieto and Cohen (12) found by in vivo experiments that intravenous infusion of probenecid or chlorthiazide causes a transient reduction in the renal uptake of fatty acids. It was suggested that fatty acids actually are substrates of the system. However, in other studies no inhibitory effect of PAH or probenecid were found on basolateral uptake of fatty acids by the isolated, nonfiltering kidney (475). Fatty acids are accumulated by kidney slices, apparently by passive binding, since the uptake does not require metabolic energy (518). It seems possible that earlier reports of a transient reduction in fatty acid uptake in vivo may have been due to displacement of fatty acids by probenecid and chlorthiazide from common binding sites. On the other hand there is evidence from microperfusion experiments that short-chain fatty acid anions are transported by a monocarboxylic acid system located at the luminal membrane (487).

We surmise from the preceding discussion that fatty acids probably are not to be regarded as true substrates of the organic anion transport system. They may interfere with organic anion transport by being bound at the translocator site, but apparently the compounds lack critical groups necessary to trigger transport. Nevertheless, introduction of aliphatic substituents into compounds with substrate capability may strongly modify transport behavior. An investigation of the properties of monoalkylated sulfamylbenzoate derivatives showed that an increase in hydrocarbon chain length from C_1 to C_5 caused an increased affinity and transport rate (430). Similar observations were made in the case of dialkyl-substrates of sulfamylbenzoate up to the di-C_3 compound (probenecid) (432). In comparison the di-C_4 compound showed increased affinity, but reduced transport rate. This phenomenon is also seen with substituted PSP dyes where a high degree of hydrophobicity is associated with low transport rates, but high affinity for the transport system (section II E).

2. Anionic Charge. A decisive determinant of transport is the presence of anionic charge that may be located on

a carboxylate, sulfonate, or sulfate or glucuronidate group (467); or it may be derived from ionization of an aromatic heterocycle as in phenylbutazone. The requirement for anionic substrate strongly suggests the presence of positive charge at the carrier binding site. Electrostatic interactions belong to the category of long-range forces (in contrast to van der Waals' interactions and hydrogen bond formation), and they are usually considered as making only moderate contributions to the binding energy of small molecules by proteins. This is seen, for example, in the interaction of the basic (bivalent anion) form of phenol red in preference to the acid (monovalent anion) form of the indicator dye to the positively charged high-affinity binding site on serum albumin (279).

Here the electrostatic interaction can be estimated to make a contribution of 0.4 kcal/mole, to be compared with about 6.2 kcal/mole for the total interaction with basic dye. There are examples in enzymatic catalysis of electrostatic interactions in which the charged groups do not participate in the catalytic process, but exert a moderate influence on the rate of reaction (240). In the case of the organic anion transport system, two possibilities may be considered to account for the pronounced effect of charge: (i) the binding site is endowed with a surplus of positive charge; (ii) the presence of negative charge on the substrate is required to trigger the translocation mechanism. The two possibilities are not mutually exclusive. According to the second possibility, one may expect the existence of uncharged analogues which are not transported but which inhibit the transport of organic anions. Few such compounds are known, but Despopoulos (119) found that a series of uncharged *N*-methylated oxypurines inhibit PAH transport. This was attributed to hydrogen bond formation between the carbonyl groups on the substrate and the carrier. Furthermore, the inhibitory effect of oxypyrimidines (121) and phenylbutazones (127) on PAH accumulation seems unrelated to their ionization state.

The formation of an ionic bond with dipole character as proposed by Despopoulos (122) presumably would require interaction with a positive charge on the carrier in a nonpolar milieu. One may question the applicability of this concept, considering the wide variety of types of anionic groups that are substrates, including compounds with delocalized anionic charges. Also ion-bond formation would entail stricter steric requirements in relation to other critical groups on the substrate which would make it even harder to establish common binding criteria for the various substrates.

While the presence of anionic charge is usually a prerequisite for transport, this is not always the case. Some aromatic amino acids, in zwitterionic form, such as L-tryptophan (521) and L-tyrosine (522) are substrates; see table 1, group IX. In the frog kidney, a specialized system exists for secretion of urea, which is inhibited by PAH and probenecid (165). Creatinine is secreted in many mammalian species by a probenecid-sensitive mechanism (501). On the other hand, there is evidence that the dicarboxylic anions may interfere with the organic anion system, while themselves being transported by another system (420). The latter is also different from that of the monocarboxylic acid system which is involved in the transport of glutamate and L- and D-lactate (328).

3. A Model for Substrate Specificity. On the basis of the preceding discussion it is apparent that organic anion substrates exhibit several interactions with the receptor site of the carrier on the basolateral membrane, including: a) binding of an aromatic group, b) hydrogen bonding via substrate carbonyl groups, c) electrostatic interactions, and d) unspecific hydrophobic interactions. Stricter steric requirements presumably must exist for binding of the aromatic group and hydrogen bond formation than for electrostatic and hydrophobic interactions. In this section we try to pursue the question of substrate specificity further by presenting a speculative model for substrate-receptor interaction (fig. 7). The carrier receptor site incorporates a binding site for an aromatic group and three neighboring groups that are capable of forming hydrogen bonds with substrate carbonyl groups. We first consider four compounds for which

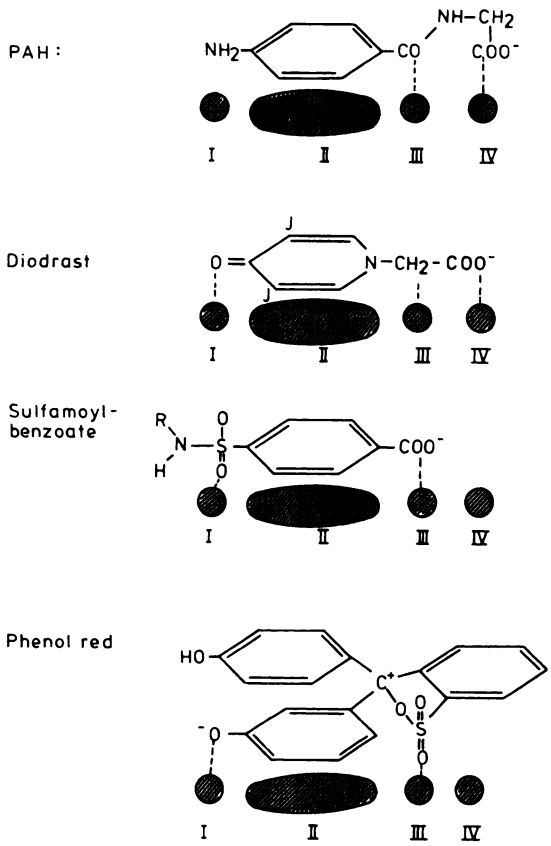

FIG. 7. Model for the receptor site of the hippurate system. Binding area II is the aromatic binding site, and binding areas I, III, and IV represent groups with a potential for hydrogen bond formation with carbonyl groups of the substrate (indicated by broken lines). Note that electrostatic and hydrophobic interactions are not indicated in the figure, since they are considered to be unspecific and have fewer steric requirements.

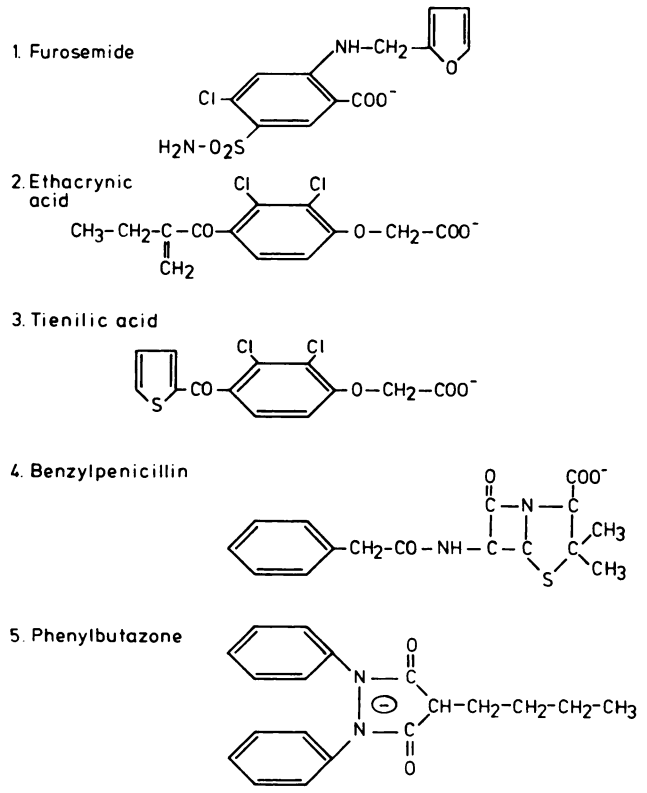

FIG. 8. Constitutional formulas of drugs that exhibit high affinity for organic anion secretion.

there is fairly good evidence from clearance and in vitro experiments that they are transported by the hippurate system: PAH, diodrast, phenol red, and monoalkyl-substituted sulfamylbenzoate (90, 430, 443).* For all substrates a three-point attachment of the substrate to a receptor site can be identified, in addition to electrostatic interactions. Since methylsulfamylbenzoate is a moderately good substrate (430), we may tentatively conclude that a three-point attachment is the minimum required to obtain a high affinity substrate. We may then proceed by applying this conclusion to the drugs shown in figure 8. Among these ethacrynic acid, tienilic acid, furosemide, and phenylbutazone have the potential for high affinity interaction with the carrier. The latter two compounds may only be able to form one hydrogen bond, but binding affinity presumably is supported by hydrophobic interaction. Furthermore, the model predicts the possibility of low affinity binding with pyrazinoate and salicylate, in agreement with experimental findings. The precise basis for the high affinity interaction of benzylpenicillin with the transport system is more difficult to delineate. Part of the molecule is analogous to that of hippurate, but the penicillate moiety, in addition to the carboxylate group also has a carbonyl group that may be essential.

The speculative nature of the model is recognized, but nevertheless it serves to emphasize that correlations between transport properties on the one side and chemical structure and physicochemical properties on the other side are probably within reach. As mentioned previously acylglycines act as substrates, but a rather long hydrocarbon chain is required. Heptoylglycine is a high affinity substrate, whereas an inhibitory effect of buturyl- and acetylglycine on PAH transport is barely detectable (271, 407). Accordingly secretion of this type of compound is probably assisted by hydrophobic interaction, and a three-point attachment to the receptor site model of figure 7 can be visualized.

C. Development and Substrate Induction

The capacity of renal cells of most species to actively secrete organic anions at birth is low, but increases rapidly in the neonatal period (230, 331, 382). Hook et al. (230) found that the slice-medium value of PAH reached a maximum in cortex slices from 4-week-old dog puppies and then declined to adult values. However, in clearance and micropuncture experiments PAH secretion steadily increased for 8 weeks after birth and had not attained adult values during this period (230, 231). In studies on microperfused straight tubules Schwartz et al. (410) found an appreciable increase in the capacity for PAH secretion per unit of tubule length; this showed that the increase in PAH secretion is not solely due to an increase of renal mass. A progressive increase in PAH accumulation with age was also observed by studying uptake of PAH in isolated tubules from rabbit kidney (140). Therefore, the high accumulation ratios obtained after 2 weeks with kidney slices seem to reflect a peculiarity of this assay system.

Hirsch and Hook (215–217) found that, during development, PAH secretion may be stimulated by administration of penicillin and other organic anions. This was considered to be the result of an increased biosynthesis of carrier protein, since substrate induction was accompanied by an increased incorporation of ^{14}C-leucine into protein in renal homogenates. Furthermore, both substrate induction and protein synthesis were inhibited by cycloheximide (217, 462). There is some disagreement as to which conditions are necessary for substrate induction. Hook and collaborators maintain that substrate induction is only seen during maturation (352), but in the experiments of Stopp et al. (462) substrate induction was observed only after administration of organic anion to adult rats. However, substrate induction could be elicited during recovery after administration of nephrotoxic agents like potassium dichromate and glycerol (64).

Hewitt et al. (212) attempted to use the pattern of development and substrate induction in rabbit kidney to delineate various subsystems of organic anion transport. Pretreatment with penicillin or probenecid resulted in stimulation of PAH, penicillin G, and phenol red accumulation, whereas pretreatment with urate, sulfisoxazole, acetylsalicylate, and chenodeoxycholate had no effect on

* However, Tanner et al. (469) consider it possible that phenol red may be secreted by a different carrier, since secretion of the dye cannot be demonstrated in the frog, whereas PAH and diodrast are secreted in this as in other species.

organic anion accumulation. Furthermore, urate accumulation, unlike that of PAH, was not discernible until 2 weeks after birth. On this basis it was concluded that urate is secreted in a different way than PAH, phenol red, and penicillin; this is in contrast to the conclusion drawn from other investigations (section II B). The development of chenodeoxycholate transport seemed to follow a pattern that was different from that of both PAH and urate, and this compound was considered to be transported by a third subsystem. Some aspects of the conclusion concerning the relation between urate and PAH accumulation deserve comment. First, accumulation of urate was lower than that of PAH, in accordance with other studies (260, 357, 426), but the rise of urate uptake (in relative terms) during development was quite similar to that of PAH. The failure to observe intracellular accumulation until 2 weeks after birth could have been due to concomitant reabsorption of urate (322). Secondly, substrate induction is not a specific phenomenon. Kluwe et al. (267) found similar increases in PAH accumulation after challenge with 3-methylcholantrene and phenobarbital, which are recognized as stimulators of various drug metabolizing enzymes. Bräunlich and Kangas (65) point out that good inducers of organic anion transport are characterized by pronounced protein binding. This may account for the failure of urate to act as an inducer of transport. Accordingly, the strongest argument in favor of differences in urate and PAH transport in the study of Hewitt et al. (212) is the lack of induction after challenge with probenecid or penicillin.

D. Reabsorption of Secreted Organic Anions

1. Reabsorption by Passive Processes. In many cases the existence of tubular secretion is masked by tubular reabsorption. There are several driving forces that favor tubular transfer of organic anions in the reabsorptive direction in the proximal and distal tubule: a) the presence of large concentration gradients between tubule fluid and peritubule fluid, resulting from reabsorption of water; b) the transcellular electrical potential difference, which is slightly negative in the proximal tubule fluid (72, 180) and more negative in the distal tubule fluid (72, 186), favors reabsorption of anions from the tubule fluid; and c) the pH difference between tubule fluid and peritubule fluid, which is negative, except during alkalosis, favors reabsorption of the corresponding (protonated) acid. Transcellular permeation is determined as the product of these driving forces and the permeability coefficients of the anion and the corresponding acid across the membrane. However, in the proximal tubule, a large fraction of the tubule fluid is reabsorbed hydraulically by the paracellular route (54, 180). In addition to water, the tight junctions appear to be permeable to Na^+, Cl^-, HCO_3^-, urea, and other low molecular weight substances (180, 484). But the tight junction presumably only has a restricted permeability to compounds with a larger molecular weight like glucose (489), amino acids (489), and PAH (488). Nevertheless, some organic anions have a high permeability, e.g. salicylate (508), probenecid (509), and other aromatic anions (e.g. 153, 156, 274, 505). It is very likely that passive reabsorption of such compounds occurs by non-ionic diffusion of the undissociated form across the lipid matrix of the plasma membranes. This mode of reabsorption is favored for lipophilic compounds with weak acid properties, i.e. with a pK_a that is not too far removed from that of the tubule fluid (315, 501, 506). However, several observations caution against attributing passive reabsorption of organic anions solely to non-ionic diffusion. In a micropuncture study of salicylate excretion in the rat, experimentally induced pH changes of tubule fluid in the proximal tubule affected salicylate reabsorption to a smaller extent than expected from reabsorption by non-ionic diffusion (387). Despopoulos and Segerfeldt (128) found that efflux of a variety of organic anions from rabbit kidney slices was of a comparable magnitude and unrelated to lipid solubility. The high accumulation ratios observed for probenecid in rabbit kidney slices, relative to the initial uptake rate (431), also argue against the existence of a high rate of non-ionic backdiffusion across the basolateral membrane. Phenolsulfophthalein dyes are reabsorbed from tubule fluid (183, 504), and, in the case of phenol red, the extent of reabsorption in the rabbit kidney is sensitive to urinary pH changes (183). For this substance, reabsorption by non-ionic diffusion is rather unlikely, because of the low pK_a value of the indicator dye. It was suggested that the acidic form (monovalent anion form) is reabsorbed in preference to the basic form (bivalent anion form) of the indicator dye (183). A possibility that has not been appropriately explored is that reabsorption of many organic anions across the plasma membranes of the proximal tubule may occur through a hydrophilic pathway, e.g. mediated by anion exchange proteins. In this connection, attention is drawn to the fact that in most experiments urinary pH has been changed by alkalinization during infusion of bicarbonate which conceivably could lead to inhibition of reabsorption by competition for anion-exchange.

In comparison to the proximal tubule the permeability of the distal tubule to organic anions appears to be smaller. This has been verified by systematic comparison of efflux of various organic anions from the proximal and distal tubule in microperfusion experiments (342, 446). Stop-flow experiments support the view that this segment of the nephron is relatively impermeable to organic anions whose reabsorption is not affected by urinary pH, e.g. of urate (23, 317, 318), PAH (299), pyrazinoate (507), and *m*-hydroxybenzoate (311, 312). However, in the micropuncture study of Roch-Ramel et al. (390) reabsorption of salicylate was prominent and probably could be accounted for by non-ionic diffusion.

Detailed information regarding the permeability characteristics of the remaining segments of the nephron to organic anions is not available at present. The major part

of the evidence, based on micropuncture data, favors the view that these are permeable to urate to a very limited extent (389), although significant reabsorption of urate has been suggested to occur in the loop of Henle in the rat (190), and, to some extent, in the distal part of the nephron (281). PAH may be reabsorbed to a limited extent both in the loop of Henle and distal part of the nephron (9). The operation of the medullary countercurrent system could favor reabsorption in appropriate cases. An example is provided by salicylate where distinct reabsorption was demonstrated during hydropenia between micropuncture collection sites in the proximal and distal tubule (390).

2. Reabsorption by Active Processes. Among the organic anions assembled in table 1 there is clear evidence for reabsorption by carrier-mediated mechanisms in the proximal tubule for the following compounds: urate, PAH and diodrast in the Necturus, *m*-hydroxybenzoate, pyrazinoate, L-tryptophan, L-phenylalanin, ascorbate, bile acids, and thiosulfate. In addition a reabsorptive system has been demonstrated to exist for nicotinate (55, 97).

Criteria for establishment of active transport were discussed in connection with urate reabsorption (section II B) and these include the following points: a) saturatability of net reabsorption at high plasma concentrations, b) transport against an appreciable concentration gradient (concentration in tubule fluid smaller than in the peritubule fluid), c) inhibition by other organic anions, d) ancillary evidence arguing against reabsorption by other mechanisms such as independence of excretion on urinary pH and diuresis, and e) studies on the uptake of the compounds by brush-border vesicles.

Micropuncture experiments and studies on the uptake by brush-border membrane preparations suggest Na^+-dependent transport for the following compounds: L-tryptophan and L-phenylalanine (434), ascorbate (473), thiosulfate (490), and taurocholate (524). The mechanism of reabsorption appears to be fundamentally different from that for secretion as indicated by the absence of inhibitory effects by representative substrates of the organic anion secretory system such as PAH, probenecid, and carinamide. The specificity pattern is also much more differentiated than in the case of the organic anion secretory system. For example, both L- and D-tryptophan are secreted, but only L-tryptophan is reabsorbed (521, 522); thiosulfate reabsorption is specifically inhibited by sulfate (26, 490); taurocholate reabsorption is inhibited by chenodeoxytaurocholate (19) and glycocholate (524); transport of ascorbate is inhibited by erythorbate, an isomer of ascorbate (473); and nicotinate transport is inhibited by the isomeric compounds picolinic and isonicotinic acid, and by pyrazinoate and benzoate, but not by structurally similar dicarboxylic acids (55). In the latter case, it is of interest to note that the Na^+-dependent transport system for L- and D-lactate (13) and other monocarboxylate metabolites (328) is different from the transport system for di- and tricarboxylic acids (244, 258, 261, 278, 530, 531). There are no known inhibitors of the reabsorption of *m*-hydroxybenzoate. Only in the case of urate reabsorption, and of PAH and diodrast reabsorption in Necturus (253, 257, 271), has it been possible to demonstrate common inhibitors for reabsorption and secretion. Nevertheless, there are both quantitative and qualitative differences between the susceptibility of the secretory and reabsorptive processes for the latter compounds to inhibition by various agents. For example, administration of diodrast (53) and probenecid (423) to man produces distinct urosuria, whereas administration of PAH leads only to slight inhibition of urate reabsorption (52). Furthermore, urate reabsorption in man is strongly inhibited by the nitrogenous base, zoxazolamine (79).

In conclusion, it is evident that the specificity pattern for reabsorption of organic anions is based on different principles and is much more diversified than for the organic anion secretory system. The relation to reabsorption of other anions which are not secreted such as amino acids, lactate, and Krebs cycle intermediates is virtually unexplored at the present time. Our ignorance in the field of organic anion reabsorption may change dramatically in the coming years, especially with the use of membrane vesicles in the study of transport properties.

IV. Summary

Anion transport systems occur in several tissues of epithelial origin such as kidney, liver, plexus choroideus, and uvea. The renal system is responsible for removal of endogenous and exogenous compounds that are not easily degraded by the body. Exogenous compounds include drugs such as probenecid, salicylate, most diuretics, phenylbutazone, pyrazinoate, and antibiotics with a penicillic acid nucleus. Excretion often occurs after conjugation with glycine, sulfate, or glucuronidate. Renal secretion of organic anions is confined to the proximal tubule and is often, but not always, most intense in the S_2-segment, depending on the organic anion and species under study. Many of the compounds are simultaneously reabsorbed, either by non-ionic diffusion or carrier-mediated mechanisms.

Current research focuses on the excretion of PAH and urate. Transport of PAH against an electrochemical gradient occurs at the basolateral membrane, whereas, transfer across the luminal membrane is down-hill, but probably carrier-mediated. Na^+ plays an important role for PAH transport, probably by a cotransport mechanism. The energy dependence of active PAH transport in many species is closely correlated with Na^+-K^+-ATPase activity. But, it seems that the thermodynamic energy inherent in the Na^+ gradient is not sufficient to account for all of the intracellular PAH accumulation, and other possible energy sources, including direct utilization of metabolic energy or exchange with endogenous anions, are under investigation.

Interesting and complex relations have been found

between urate and PAH secretion. In some species (rabbit, chicken), urate competes with PAH for secretion, but the purine is apparently secreted by a system other than PAH in primate species (pyrazinoate sensitive) and snakes. Microperfusion of isolated tubules and other in vitro studies indicate an active uptake of urate across the basolateral membrane in species with predominant secretion of urate. In the rabbit the energy dependence and effect of Na^+ and of metabolites on urate secretion is the same as that of PAH. However, some differences between the mechanism of urate and PAH secretion are noted even in this species. This especially concerns segmental differences in the ability to secrete urate and PAH, the K^+ dependence of intracellular urate accumulation, and the lack of stimulation of secretion after substrate induction of the organic anion system in the developing animal. Reabsorption of urate in many species occurs by a carrier-mediated and active mechanism exhibiting similarities to, but not identity with, tubular secretion. It seems probable that both the basolateral and luminal membrane is engaged in carrier-mediated secretion and reabsorption of urate. Currently under investigation is the possibility that an anion-exchange system in the luminal membrane is involved in urate reabsorption.

The tubular secretion of most other organic anions occurs in the same way as for PAH, but renal excretion is often modified by concomitant tubular reabsorption. The presence of tubular secretion potentiates the effect of drugs with a pharmacological action on the luminal side of renal cells (diuretics and some uricosurics) as well as nephrotoxic effects (e.g. of cephaloridine). A striking property of organic anion secretion is that compounds with very different chemical structures are secreted. An analysis indicates that the following properties probably are important for affinity to the transport system: a) anionic charge, b) an aromatic group, c) carbonyl groups with a potential for hydrogen bond formation, and d) hydrophobicity. The latter factor plays an important role for high affinity interaction of many drugs with the secretory system (e.g. probenecid, diuretics, and phenylbutazone) and substituted phenolsulfophthalein dyes. However, pronounced hydrophobicity also leads to a decrease of transport rate. The steric arrangement of chemical groupings within the substrate that is capable of interaction with the receptor site seems to be noncritical. This may be due, in part, to multiple modes of attachment of substrate to the receptor site and, in part, to the presence of subsystems with a somewhat different substrate specificity. In contrast, reabsorption by carrier-mediated systems exhibits much stricter steric requirements.

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