RENAL TUBULAR EXCRETION OF ORGANIC BASES[1]

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The first acceptable demonstration that the mammalian renal tubule could excrete solutes was performed by Marshall and Vickers in 1923 (54). Their experiments were performed with the acid dye phenolsulfonphthalein (phenol red, PSP). Since then it has been shown that the renal excretion of a considerable number of acidic organic compounds is accomplished by active tubular transport, as well as by glomerular filtration. Among these compounds are hippuric acid and a number of its derivatives, various sulfonphthalein dyes, pyridone-N-acetic acids including iodopyracet (Diodrast; 3,5-diiodo-4-pyridone-N-acetic acid), the penicillins, phenolsulfuric esters, glucuronides and chlorothiazide. The functional characteristics of the tubular transport process involved have been studied extensively, particularly with respect to the tubular excretion of *p*-aminohippuric acid (PAH), phenol red and Diodrast, and have been discussed in a number of excellent reviews (5, 92, 93, 94) including a very recent one by Sperber in this journal (89).

The fact that at least some organic amines and quaternary ammonium compounds can also be excreted by the renal tubules has been recognized much more recently. In reviewing the current status of knowledge of this phenomenon, the compounds concerned will be referred to collectively as organic bases, except in those instances where a distinction between amines and quaternary ammo-

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nium compounds is essential for accurate presentation. Most of the discussion will be devoted to an active transport system which mediates the transport of strong organic bases. The tubular excretion of other, mostly weaker, organic bases by a passive process of "non-ionic diffusion" will be described in much less detail since it has been the subject of a recent review by Milne *et al.* (55). Finally, the bases trimethylamine oxide, trimethylamine, urea, creatine and creatinine will be discussed briefly, but also separately since certain aspects of their behavior warrant separate classification.

I. METHODOLOGY

A brief and simplified description of the three methods used to the greatest extent in studies of the tubular excretory transport of organic bases should suffice, since details of these methods (20, 21, 72, 84, 88) and descriptions of less commonly used ones (12, 25) are readily available elsewhere.

The conventional renal clearance method has been used to study the tubular excretion of organic bases, infused intravenously at constant rates, in anesthetized or trained unanesthetized dogs. The rate of renal excretion of the base per minute during successive quantitative urine collection periods, of 10 to 30 minutes' duration, is measured while the base is infused intravenously at a constant rate. The rate of renal clearance is calculated by the conventional formula UV/P in which U is the concentration of the substance in urine, V the urine volume per minute and P the concentration of the material in the plasma. The value for P should of course be that which represents the unbound and therefore ultrafilterable portion of the base in the plasma. The degree of binding to plasma protein has been shown to be of a low order of magnitude, and relatively unimportant to the above calculations, in the case of the three strong organic bases studied most extensively in dogs, namely N^1-methylnicotinamide (NMN), tetraethylammonium (TEA) and Darstine (7, 9, 71). The clearance rate of the base is always compared with the simultaneous rate of clearance of creatinine or inulin, which serves as a measure of glomerular filtration rate (GFR), and usually and preferably also with the simultaneous rate of renal clearance of PAH at low plasma concentrations. Since low renal loads of PAH are virtually completely cleared from the plasma in one passage through the kidney, the simultaneous clearance of this material under these circumstances represents a measure of maximal clearance or "apparent renal plasma flow," except in the presence of tubular injury sufficient to permit passive back diffusion of PAH from glomerular filtrate to peritubular capillaries. The amount of the base contributed to the urine by tubular excretion can be calculated by subtracting the amount of base filtered per minute from UV, the former being the value obtained when GFR is multiplied by the concentration of (ultrafilterable) base in the plasma (P). If, during the course of a progressive increase in the infusion load of a base, a point is reached where its rate of tubular excretion becomes constant, this rate represents the maximal tubular excretory capacity (Tm) for the base in question. The actual extraction of a base by the kidney can also be measured by measuring its rate of excretion and its renal arteriovenous difference, simultaneously with that of creatinine and PAH (*e.g.*, 7, 73).

The accumulation of several organic bases in slices of mammalian kidney cortex has been demonstrated *in vitro* (20, 21, 22, 44). In such studies, the slices are suspended in a buffered salt medium containing the base and the suspension is shaken for 1 to 2 hours under oxygen. The accumulation of the base in the slice is then expressed quantitatively as the ratio of the concentration of the material accumulated in the slice to that remaining in the medium (S/M). Ratios up to 1.0 could represent simple diffusion of the base into the slice. However, those in excess of this value have been interpreted as representing active transport of the base by the cells of the renal tubule, since various behavioral aspects of the process are consistent with the behavior of the bases during their active tubular excretion by the intact kidney. The method is derived directly from those used to study the uptake

of PAH and of the acidic dye phenol red by mammalian kidney slices (6, 15). These in turn are an extension of earlier methods including that used to study the uptake of phenol red by the isolated renal tubules of the flounder (95, 102). The flounder tubule transports the dye from the suspending medium into its lumen with accumulation in the latter in concentrations much higher than that originally present in the suspending medium, and without staining of the intervening tubular cells. This movement of the dye against a concentration gradient, its susceptibility to inhibition by certain metabolic inhibitors and the fact that phenol red is rapidly excreted by the renal tubules of the intact kidney strongly support the concept that the process just described represents active transport. However, certain changes in the ionic composition of the medium can result in intracellular accumulation of phenol red in the flounder tubule *in vitro* (67a, 102). In the mammalian kidney slice, phenol red has also been reported to accumulate in the tubular lumen in high concentrations by some investigators (6), but within the cells of the proximal segment by others (30). The latter group postulates a two-step process for phenol red transport involving first accumulation in the cells and then movement into the tubular lumen. This has been carried even further in the case of PAH where it has been postulated that there are a number of steps in the transport process, including several diffusion steps, in which the active concentration of PAH within the cells is a vital intermediate phenomenon (31, 32). In any case, the accumulation of PAH and phenol red in renal slices *in vitro* is believed to represent active transport, since many of the behavioral properties of these two acids in the *in vitro* system have been shown to carry over to their tubular excretory transport by the intact kidney For example, their accumulation in the slice and their renal tubular excretion *in vivo* are both inhibited by certain metabolic inhibitors and augmented by certain substrates, *e.g.*, acetate (6, 15, 56, 57, 82, 83). Furthermore, these observations have been shown to apply to both acids in a strikingly similar manner (82, 83). It must be emphasized, however, that the accumulation of any substance in renal slices from a suspending medium *in vitro* could also represent binding of the substance to constituents of renal cells without active transport; hence it cannot be interpreted as indicative of the latter without other supporting evidence such as that just cited.

One of the first two demonstrations of the renal tubular excretion of strong organic bases was performed by Sperber who developed a method of measurement involving the renal portal circulation of the intact unanesthetized chicken (86, 87, 88, 89). Because it may be less widely known and because its extensive use by Sperber and others will necessitate repeated reference to it, this third method will be described in somewhat greater detail. The avian renal portal circulation of each kidney is derived from tributaries which drain the ipsilateral leg and pelvis. The portal system supplies blood to peritubular capillaries of the kidney without first going through the glomeruli, as opposed to a coexisting renal circulation in which the tubules are supplied by the postglomerular capillaries as in mammals. Introduction of a substance into the renal portal circulation of one kidney permits its relatively direct presentation to the renal tubules of that kidney and provides an opportunity for its tubular excretion before first traversing the systemic circulation and the glomeruli and without its prior distribution to other tissues. Thus, the amount of a transportable substance required for accurate measurement of its tubular transport is much smaller than in the conventional clearance procedure where the substance is injected into the systemic circulation. Furthermore, because the amount required is small and because much of it is excreted by the tubules before entering the system circulation, there is less opportunity for the elicitation of cardiovascular and other pharmacological and toxicological effects which may interfere with accurate measurements of tubular transport or may limit the infusion load of challenging inhibitors.

In actual practice, as applied to studies of the tubular transport of organic bases, the method may be described as follows. Unanesthetized hens are restrained upright and hydrated by crop tube. Urine is collected separately and simultaneously from the two kidneys through special cannulae secured over each of the two separate ureteral openings in the urodaeum. In earlier experiments the base to be studied was injected intramuscularly into

the thigh of one leg. In more recent experiments it has instead been injected intermittently or infused at a constant rate into the renal portal circulation of one kidney via the saphenous vein of one leg. If the injected or infused solutes are excreted by the tubular cells, they appear in excess in the urine from the kidney of the infused side when compared with the urine from the opposite kidney. If the infused material is not excreted by the tubules it reaches the systemic circulation and ultimately appears in equal concentration in the urine from both kidneys. If there is an incomplete extraction by the tubules of the ipsilateral kidney, it is the unextracted remainder which enters the greater circulation and subsequently reaches both kidneys in equal concentrations via the arterial blood. The excess excreted in the urine on the injected side (Exc_I) as compared to the opposite side (Exc_C), during any period of time, represents the amount extracted during one passage through the ipsilateral kidney. If the material has been infused intravenously, the amount infused per minute (INF) during the period of urine collection (*e.g.*, 10 or 15 minutes) can be divided into the excess excreted on the infused side per minute during the same period to yield what Sperber calls the Apparent Tubular Excretion or Extraction Fraction (ATEF), *i.e.*, $\frac{Exc_I \text{ minus } Exc_C}{INF}$ = ATEF. If this is multiplied by 100 the figure represents the percentage of infused material extracted by the tubules of the ipsilateral kidney in a single passage. If the material has been injected into the saphenous vein in a single rapid injection or has been injected intramuscularly into the thigh, the excess excreted on the injected side over a longer period of time can be divided by the total dose injected to give the ATEF value. ATEF values derived from experiments involving the three different methods of injection are not strictly comparable and that involving constant intravenous infusion would appear to be the most accurate.

Sperber studied the problem of distinguishing between passive diffusion and active tubular excretory transport in this system and presented a convincing method of analysis of the data obtained. He based his reasoning upon the fundamental concept that diffusion cannot result in a higher concentration of the substance in the fluid into which it diffuses than in the fluid from which it diffuses. In the preparation under discussion, the largest volume into which diffusion can occur is the glomerular filtrate, and the volume from which diffusion occurs is the renal plasma flow (RPF). In chickens the ratio of GFR to RPF is only about 1:13. This being the case, the maximum excess of the material appearing in the urine from the injected side by diffusion alone would be less than 10% of the injected load. Thus, tubular excretion is unequivocally indicated by an ATEF value greater than 0.1 (10%). However, since this is based on a maximum correction for diffusion, values of less than 0.1 may reflect a low but unassessable rate of active tubular excretion.

There exists, between the renal portal vein and the systemic venous circulation, a venous valve which controls the amount of renal portal blood supplying the kidney. Changes in renal portal blood flow, caused by different degrees of shunting, can alter the ATEF values of substances under study. In order to correct for this factor PAH, which is excreted by a tubular transport system different from that which excretes organic bases and which is almost completely extracted from the peritubular blood by the tubular cells, at low infusion loads, is infused along with the organic base. The ratio of the ATEF of the base to that of PAH can then serve as a more accurate measure of the amount of the organic base which enters the renal portal circuit and is excreted by the tubules on the infused side.

II. RENAL TUBULAR EXCRETION OF STRONG ORGANIC BASES

1. Strong organic bases shown to be excreted by the renal tubules of the mammalian and avian kidney. For purposes of ready reference these are listed in table 1, along with abbreviations which will be used in referring to some of them throughout this review. The compounds listed in the left-hand column occur naturally in the tissues and urine of vertebrates, while those on the right are synthetic

TABLE 1

Strong organic bases shown to be excreted by the renal tubules of the mammalian and avian kidney

guanidine	tetramethylammonium (TMA)
methylguanidine	tetraethylammonium (TEA)
piperidine	tetrabutylammonium (TBA)
N^1-methylnicotinamide (NMN)	trimethyloctylammonium
thiamine	mepiperphenidol (Darstine)
choline	tolazoline (Priscoline)
histamine	hexamethonium

chemicals. It should also be noted that some of these bases are amines while others are quaternary ammonium compounds.

In 1947 Rennick *et al.* (73) demonstrated a very rapid rate of renal excretion of intravenously injected TEA by the dog. Their suggestion that this was based on tubular excretion as well as glomerular filtration of the compound was substantiated by the fact that the renal extraction of TEA infused into the renal artery was 2 to 2½ times that of simultaneously infused creatinine. Furthermore the extraction was 81% that of PAH, infused in amounts which resulted in extraction of 85 to 90% of the infused PAH load. In the same year Sperber (86, 89, 90) first described experiments which demonstrated the renal tubular excretion of guanidine, methylguanidine, piperidine and NMN by the hen. It has since been shown that the rates of renal clearance of the following bases are 2 to 3.5 times glomerular filtration rate in the dog and may approach or equal the clearance of low tubular loads of PAH in this species: NMN (7), TEA (69), mepiperphenidol (Darstine) [1-(3-hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium] (9), and tolazoline (Priscoline) (2-benzyl-2-imidazoline) (60). Renal tubular excretion has also been demonstrated in the chicken for TEA (69), histamine (46), tolazoline (39), thiamine, choline, hexamethonium (68), Darstine (101), tetramethylammonium (TMA), tetrabutylammonium (TBA) (34) and trimethyloctylammonium (104). As already indicated, differences in methodology prevent a direct comparison of the transport rates of all of these bases in the chicken, as does the paucity of information concerning their Tm's. Using the method of constant i.v. infusion, with low infusion loads, ATEF values for PAH in some cases have been as high as 0.8 to 0.95, ATEF ratios (base/PAH) for NMN, TEA, tolazoline, thiamine and TMA being as high as 0.7 to 1.0, with intermediate values for choline and TBA, and values indicative of only slight tubular excretion for hexamethonium. Accumulation in renal slices has been demonstrated for TEA (21), Darstine (44) and NMN (20) with S/M ratios, in the absence of oxidizable substrates, as high as 8, 13 and 8 respectively.

These multiple demonstrations of the renal tubular excretion of a wide variety of organic bases raise a number of important questions concerning the nature of the process involved, to which the greater part of this review will be devoted. The major points to be considered are as follows: Is the tubular excretory process

an active or a passive one? If the former, is it mediated by a single transport system or are there different systems for different bases? Is the process mediated by the same active transport system as that which mediates the excretion of organic acids such as PAH, phenol red and Diodrast? If active transport is involved in the excretion of strong organic bases, what are the metabolic requirements of the transport system? What is the nature of the surface or intracellular receptor(s) or carrier substance(s) involved? Since various items of experimental evidence bear upon more than one of these questions, the order of their presentation will not coincide with the above.

The rate of renal clearance, in dogs, of the secondary amine and strong base mecamylamine (Inversine, 2-methylaminoisocamphane), is rapid and may equal that of PAH when the urine is acid, but is below GFR when the urine is alkaline (1). Such behavior has not been reported for the other strong organic bases listed in table 1 but has been demonstrated for a number of weaker organic bases (55, 61). Furthermore, the ATEF value of mecamylamine in chickens, even when the urine is acid, is very low (< 0.1 or 10%) (96, 100); hence mecamylamine is discussed along with the latter group of bases in a later section of this review.

2. Evidence that the tubular excretion of strong organic bases involves active transport. At least some of the strong organic bases are virtually completely cleared from the plasma in one renal circulation in the dog. Since they are believed to exist in the plasma and urine in only one form, namely as positively-charged ions, it is difficult to visualize their tubular excretion by any mechanism other than one involving their transport against a concentration gradient. If the accumulation of strong organic bases in renal slices in high concentration, from a relatively low concentration in the suspending medium, is analogous to tubular excretion *in vivo*, this likewise involves a gradient-opposed concentration process. Furthermore, Sperber's arguments that ATEF values in excess of 0.1 (10%) could not occur in the chicken on the basis of passive diffusion but only by active transport against a concentration gradient are convincing (see METHODOLOGY). In terms of established concepts of renal physiology such transport should be an active process, probably dependent upon metabolic energy and upon transient combination of the transportable compound with "receptor" or "carrier" elements in the cell or on its surface. Some component or components of such a system should be rate limiting so that the system should have a maximum transport capacity (Tm) for the compounds, and if two compounds were presented to the tubular cells simultaneously for transport in adequate amounts, mutual depression of the excretion should occur on a competitive basis (5, 81, 83, 84, 94).

In the case of tubular excretion of strong organic bases, the largest amount of evidence in support of active transport relates to the fact that various bases have been shown to depress the simultaneous tubular excretion of others and that, in the renal slice at least, the inhibition appears to be a competitive one. In some instances, studies with a given pair of organic bases have permitted the demonstration that each can inhibit the transport of the other and these are

cited first, as most important to the question at hand. Darstine has been shown to produce a rapid and readily reversible inhibition of the tubular excretion of NMN in the dog (40). The reverse inhibition was also demonstrated when sufficiently large infusion loads of NMN were employed (40). Similar mutually inhibitory relationships have been demonstrated with NMN and TEA in the chicken (34) and in the mammalian kidney slice (20, 22).

Other examples of inhibition of tubular excretion of one organic base by another base known to be excreted by this pathway are as follows: In his original experiments Sperber (86, 89) showed that the tubular excretion of piperidine was depressed by simultaneous administration of NMN in the chicken. Subsequent experiments with this species in other laboratories have shown that TBA, trimethyloctylammonium, Darstine and tolazoline all can inhibit the tubular excretion of NMN (34, 39, 101, 104), that NMN can inhibit the tubular excretion of TMA (34), that tolazoline can inhibit the tubular excretion of histamine (47) and that thiamine can inhibit the tubular excretion of choline (68).

In renal slices, accumulation of TEA has been inhibited by addition, to the suspending medium, of appropriate amounts of tolazoline, guanidine, methylguanidine, and piperidine (22). Similarly, the uptake of NMN by slices has been inhibited under appropriate conditions by tolazoline, Darstine, choline, guanidine and methylguanidine (20).

The fact that two bases can mutually inhibit each other's excretion by the renal tubule or each other's accumulation in renal slices strongly suggests that the inhibition involved represents competition for tubular excretion by an active transport system. However, the mere fact that inhibition of NMN transport in the chicken (34, 101) and inhibition of accumulation of NMN and TEA in slices (20, 22) by various other transportable bases was proportional to the concentration of the inhibitor, within the effective range, is not sufficient evidence to confirm this hypothesis. Attempts to reverse inhibition of the tubular transport of one base by another, in the dog and the chicken, through the procedure of increasing the infusion loads of the inhibited base have not been reported. However, in their studies of the accumulation of NMN in renal slices, Farah *et al.* (20) found that increasing the concentration of NMN necessitated an increase in the amount of TEA, Darstine, tolazoline and choline required to maintain a 50% inhibition of NMN uptake. This observation does support the concept that inhibition of the transport of one base by another is a competitive phenomenon. In the same study, additional evidence for competitive inhibition was presented in Lineweaver-Burk plots derived from experiments in which the inhibition of NMN uptake by slices was studied over a wide range of concentrations of *both* NMN and a number of transportable inhibitory bases. It must be acknowledged that the system in question here is a complex one involving whole and intact cells, *i.e.*, one in which factors of permeability and diffusion are involved. Hence it is one in which limiting factors other than the direct reaction of a substrate (*i.e.*, a transported base) and an inhibitor with a transport receptor may operate and may do so to different extents at different concentrations of the reactants. Thus, such plots cannot be interpreted with as great a degree

of confidence as can plots derived from studies of biochemical reactions in cell-free or purified enzyme systems (16, 48). Nevertheless, the plots can be accepted as a supporting argument for the concept that the ability of a pair of organic bases to inhibit each other's accumulation in a renal slice is a competitive phenomenon.

Evidence that the tubular excretory transport of organic acids, such as PAH, phenol red and Diodrast, and the reabsorptive transport of glucose are rate limited, *i.e.*, that there is a definite Tm for these substances, is very adequate, particularly with respect to the dog and man (5, 81, 84). Similar evidence for a maximal tubular excretory capacity or Tm in the case of strong organic bases is much less extensive. This is partly because, in the case of many of them, large infusion loads designed to saturate the transport capacity of the tubules have evoked pharmacological responses, such as alterations in systemic and renal vascular dynamics, which interfered with accurate measurements. Rennick has presented evidence for the existence of a Tm for TEA in the anesthetized dog (69). Her experiments demonstrated that an increase in systemic intravenous infusion loads of TEA resulted in a progressive decrease in the ratio of the clearance of TEA to glomerular filtration rate, the latter remaining constant within narrow limits, and that the amount of TEA transported became relatively constant at a level of 1 to 1.4 mg per min per m^2.

In three different experiments in unanesthetized dogs we found that the tubular excretory rate of N^1-methylnicotinamide became relatively constant at higher plasma levels (40, 62, 63) but the results were sufficiently variable to preclude the conclusion that a Tm was definitely reached. The limited data published on the tubular excretion of tolazoline at increasing infusion loads likewise suggest, but do not definitely establish, the existence of a Tm for this substance (60). Sperber (91) has published a value for the Tm of NMN in the chicken. His experiments were performed by injection of doses of NMN of differing magnitude into the renal portal circulation of one kidney, each over a short interval (1 min), and subsequent separate collection of urine from the two kidneys for 15 minutes. The Tm values obtained (5 μmol/min for the whole animal) was slightly lower than the corresponding value for PAH (7 μmol/min). Sperber refers to this method of determining Tm as "an approximate one" and indicates certain factors which make a strict interpretation of results difficult. In subsequent studies he was not able to establish a Tm for histamine within the limits of the infusion loads employed (47). We have attempted to establish a Tm in the chicken for NMN during its constant intravenous infusion into the renal portal circulation at progressively increasing tubular loads (96). These experiments have not been successful because of the progressive increase in the amount of NMN which escapes into the general circulation for excretion by the tubules and glomeruli of both kidneys, a factor which again makes the interpretation of data extremely difficult.

Cross and Taggart (15) have shown that the amount of the organic acid PAH which accumulates in slices of rabbit kidney cortex decreases relative to the amount in the surrounding medium as the latter is increased and that at higher

concentrations this discrepancy becomes more manifest. This is interpreted as reflecting an approach toward saturation of the transport system for PAH in the slice. A maximal ability of the slice to concentrate PAH has also been reported by others (*e.g.*, 32). A decrease in the S/M ratio for the base Darstine has been reported with slices of rabbit kidney cortex as the concentration of the base in the suspending medium was increased ten-fold (44). However, data concerning the proportionality of this decrease in S/M ratio to the concentration of Darstine in the medium and concerning the actual amount of Darstine accumulated at these and numerous intervening concentrations were not presented; hence it is not possible to interpret this finding definitely as a saturation of the transport mechanism for Darstine. Unpublished data from Farah's laboratory have failed to reveal an absolute or approaching saturation of the mechanism for uptake of NMN by slices of dog kidney cortex when the concentration of NMN in the medium was progressively increased.

In summary, it can only be stated that the evidence for the existence of a Tm for various organic bases is relatively limited and that further studies are needed.

As Taggart has pointed out (94), the fact that a transport mechanism can be depressed by unfavorable metabolic conditions provides only suggestive evidence that the transport process is an active one, directly dependent upon metabolic energy, since such conditions may affect cellular integrity and permeability on a broader basis. Experiments with organic bases along these lines, presented in a later section of this review, must therefore be interpreted conservatively.

Thus, evidence that the tubular excretion of strong organic bases is mediated by an active transport system is currently based on the fact that tubular excretion occurs against a concentration gradient under certain circumstances and that bases excreted by this pathway can depress each other's excretion by a mechanism which is almost certainly a competitive one.

3. Specificity of transport of strong organic bases. Numerous items of evidence indicate that the tubular transport of strong organic bases is mediated by a transport system (or systems) distinct from that which mediates the transport of PAH and phenol red, and hence of other organic acids of this group.

Sperber (89, 90) demonstrated that the tubular excretion of NMN, methylguanidine, guanidine and piperidine in chickens was not influenced by the intravenous injection of doses of hippuric acid and Diodrast which were adequate to depress the tubular excretion of a number of glucuronides and phenol sulfuric esters, including hydroquinone sulfuric esters. On the other hand, large doses of NMN which effectively inhibited the transport of piperidine did not alter the transport of hydroquinone sulfuric ester nor of phenol red (89, 91).

In dogs the transport of NMN was not altered by maximally transportable loads of PAH nor was the Tm of PAH altered by simultaneous infusion of NMN (7) though the adequacy of the infusion loads of the latter are difficult to assess since its Tm is not known. Carinamide (7) and probenecid (Benemid) (8) in amounts which very effectively inhibit the transport of PAH, did not decrease the transport of NMN. The tubular transport of tolazoline was shown to be unaffected by infusions of PAH which saturate the transport system for the

latter (60). The transport of Darstine has likewise been shown to proceed independently of the simultaneous maximal transport of PAH and to be unaffected by probenecid (9). Large infusion loads of PAH, sufficient to exceed its Tm, did not inhibit the simultaneous transport of TEA, at infusion loads designed to exceed its Tm, and doses of probenecid which depressed the Tm of PAH 90 to 100% had only a very slight effect on the simultaneous transport of TEA (71).

The tubular transport of NMN in the dog has been completely and very rapidly blocked by small single intravenous doses of a basic cyanine dye, 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride (#863), for prolonged periods of time (63). The clearance of PAH and the Tm's of PAH and glucose were not altered by the dye.

The tubular transport of low infusion loads of NMN has been blocked in the chicken by a large number of inhibitors at infusion loads which did not block the simultaneous transport of PAH. Such experiments are less critical than those just cited with respect to demonstrating specificity, since the infusion loads of PAH, like those of the base, were small in these experiments and were intended to measure relative renal portal flow rather than the Tm of PAH (34, 39, 72, 101).

In renal slices, the S/M ratio for TEA has been depressed readily by addition to the suspending medium of concentrations of tolazoline, guanidine, methylguanidine, NMN and piperidine which had little or no effect on the simultaneous accumulation of PAH in the slice (22). Accumulation of PAH and TEA occurred simultaneously with no mutual interference. Also the transport of PAH in slices was readily inhibited by concentrations of penicillin and probenecid which did not depress the S/M ratio for NMN (20). The accumulation of Darstine in renal slices was also not depressed by high concentrations of carinamide and PAH (44).

The fact that certain metabolic inhibitors and oxidizable substrates have different qualitative or quanitative effects on the tubular transport of strong organic bases on the one hand, and of PAH and phenol red on the other is discussed in a later section of this review.

In one study the optimal temperatures for the accumulation of PAH and organic bases in renal slices were shown to be different (20). Under the experimental conditions employed, using a concentration of 1×10^{-4} M of PAH and NMN or TEA in the suspending medium, a maximum S/M ratio of slightly over 8 was obtained at the end of 2 hours when the temperature of the bath was 25°C in the case of PAH; a similar maximum for the bases occurred at 37°C, a temperature at which the uptake of PAH had already begun to decline. The pH optimum in these studies was found to be 6.5 for uptake of PAH and 7.5 to 8 for that of NMN.

When all of the evidence presented is examined in aggregate, the conclusion that the transport of organic acids such as PAH and phenol red on the one hand and the transport of strong organic bases on the other are mediated by transport systems which are functionally distinct with respect to at least one component seems completely justified.

The strong organic bases exist at the pH of blood and body fluid as cations.

For this reason, the question arises as to whether their tubular transport is related to that of the inorganic monovalent cations and particularly to that of potassium since potassium is excreted by the renal tubules through ion exchange (3, 4, 43, 58). Several interesting preliminary reports have suggested a relationship between organic cation transport and that of potassium (17, 38) but the question remains unsettled at this writing. The fact that the organic cations are probably excreted at the level of the proximal tubule (70) while tubular excretion of potassium is at the distal segment (3, 4) would argue against such a relationship. However, the possibility should not be excluded that the transport of organic cations in an excretory direction and that of sodium and potassium in a reabsorptive direction in the proximal segment could involve common components of different transport systems or possibly even the same system. In this regard it is interesting that the mercurial diuretics meralluride (Mercuhydrin) and mersalyl (Salyrgan) inhibited the tubular transport of TEA in the dog under conditions which did not effect the Tm of PAH (69, 71). This inhibition was reversed by two monothiols, cysteine and gluthathione, and the dithiol dimercaprol (BAL). In the experiments with Salyrgan the simultaneous increase in sodium and water excretion was reversed only by the dithiol. The tubular excretion of potassium is also inhibited by the mercurial diuretics (4, 58). The possible relationship between tubular transport of organic and inorganic cations appears to be a fruitful area for future investigation.

Some consideration has been given to the question of the specific site of transport of organic bases by the renal tubules. The fact that both PAH (27) and TEA (29) can be excreted by the kidney of the aglomerular fish, *Lophius americanus*, suggests the proximal convoluted tubule as the site of such transport in mammals, since the tubules of these fish have brush border cells histologically similar to the cells of the proximal tubule in mammals (25). A relatively new technique for localization studies is the method of stop-flow analysis (51) in which the exteriorized ureter of one kidney of an anesthetized dog is occluded during mannitol diuresis and, upon release, successive fractions of urine are collected at intervals of a few seconds for several minutes. During this time the successive fractions show differences in composition which reflect the stratification of the urine in the nephron and the sites at which various tubular events occur. In such studies the site of tubular excretion of PAH has been fixed at the level of the proximal tubule (51) and in one additional preliminary report the same segment of the tubule has been proposed as the site of transport of TEA (70).

4. The question of a single transport system or multiple systems for the tubular excretion of strong organic bases. The various strong organic bases known at this time to be excreted by the renal tubules have been listed. The ability of many of these to inhibit the transport of others and mutual inhibition with certain pairs have been demonstrated and described above (Section II, 2), though all possible combinations have not been studied in order to establish a relationship in their transport.

There can be added to the findings already presented the fact that, in the dog,

cyanine dye #863 inhibited the tubular transport not only of NMN and TEA but also to a lesser extent that of Darstine (40) and that the dye also inhibited the tubular transport of NMN (101), TEA (72), and thiamine (68), in the chicken.

When all of these factors are considered and after addition of indirect reasoning in the case of inhibitory relationships not yet studied, there seems little reason to doubt that at least one cellular component or receptor substance in tubular cells is essential to and participates in the transport of all the strong organic bases listed in table 1 of this review. Furthermore, it seems safe to assume that each of the bases mentioned could inhibit the transport of all others under appropriate experimental conditions in one or more test systems.

In a few instances authors have reported failure to demonstrate inhibition of the transport of one strong transportable base by another; *e.g.*, tolazoline and TEA by NMN in the dog (60, 71), Darstine by NMN in the slice (44) and TEA by TMA in the slice (22). These can now be explained in most cases on the basis of the widely differing affinities of various bases for transport (see below). A base with a weak affinity for the transport system may not be able to inhibit the transport of bases with a significantly greater affinity for the system under certain experimental conditions. The inhibition may, however, be demonstrable under other conditions, for example, at a different ratio of the concentrations of the pair of bases under study, or in another test system. The reverse inhibition may be demonstrated with ease in all test systems. In a few instances, failures to demonstrate inhibition in the dog or chicken have also been based on the limitation imposed upon the size of infusion loads of the inhibitor by its toxicity; *e.g.*, NMN by TMA in the chicken (34). Such a limitation can be expected to complicate clearance studies with dogs more frequently than studies with chickens and renal slices since the infusion of pharmacologically active bases into the systemic circulation of dogs will more readily evoke complicating responses. In still other instances, interference by one base in the analytical procedure for the other makes it impossible to study the relationship between pairs of compounds. It should also be recognized that, due to limited existing knowledge of the Tm's of organic bases in the dog and the chicken, inhibition studies usually have not been based upon experimental conditions which are definitely known to involve saturation of the transport system. Rather, they have depended largely upon the examination of the relationship over a wide range of arbitrarily selected infusion ratios of the challenging base to the base which is being measured, the infusion load of the latter being maintained at a low, constant level.

5. Relative affinities of various strong organic bases for their tubular transport system, including structure-activity relationships. Considerable information along these lines has been obtained if one assumes that the relative abilities of a series of bases to inhibit the transport of a selected single base (*e.g.*, NMN) reflects the relative affinities of the inhibitors for the transport system involved. The renal portal test system of the chicken and the renal slice method have yielded the most accurate information, because of the relatively high degree of reproducibility of quantitative measurements in these systems under standardized experimental conditions.

In studies with chickens the following procedure was used in the reviewer's laboratory (34, 101). NMN and PAH were infused simultaneously into the renal portal circulation of one kidney for four successive ten-minute periods and then for four additional periods after an immediate switch to an infusion containing an additional challenging base. Since inhibition, when it occurred, usually reached a maximum in ten minutes, the average ATEF ratio of NMN/PAH for the four control periods was compared with that obtained during the last three experimental periods, the first period of (developing) inhibition (*i.e.*, period 5) being omitted from the calculation. The concentration of NMN in the infusion was kept constant from experiment to experiment, the infusion load being 0.17 μmol/kg/min. The concentration of the challenging base was varied, the molar infusion ratio of the challenging base to NMN in different experiments ranging from 0.25:1 to 30:1. Under these conditions the degree of inhibition of NMN transport by a given base yielded a reproducible dose-response relationship and the inhibitory potency of various bases, *i.e.*, their affinity for the transport system, differed widely. The latter is illustrated by the fact that Darstine infused with NMN at a molar infusion ratio (Darstine:NMN) of 0.25:1 produced an inhibition of NMN transport of approximately 60%, that TEA produced a comparable degree of inhibition of NMN transport when infused at a molar concentration 30 times that of Darstine and that choline, infused at a molar concentration 120 times that of Darstine, produced an inhibition of only 35%. On the basis of such data, the relative affinities of a number of transportable bases for the transport system could be listed in order of decreasing affinity as Darstine, TBA, trimethyloctylammonium, tolazoline, TEA and choline. Maximally tolerated infusion loads of TMA did not inhibit NMN transport, even though such loads were greater than loads of TEA which produced a 60% depression of NMN transport. However, very large infusion loads of NMN depressed the tubular transport of both TEA and TMA when the latter two bases were infused at the rate of 0.17 μmol/min. These last two findings make it possible to place both TMA and NMN below TEA in the series.

In another laboratory quantitative comparisons have been made of ability of a number of transportable bases to inhibit the accumulation of NMN in slices of dog kidney cortex (20). On the basis of an inhibition index, which was the concentration of inhibitor producing a 50% depression of NMN uptake, the bases studied could be listed in decreasing order of potency as Darstine, tolazoline, TEA, choline, methylguanidine and guanidine, the latter two being approximately the same. It is interesting and reassuring that this order is the same as that found in the chicken, in the case of those bases studied by both methods. Again the ability of various bases to inhibit NMN transport differed greatly, Darstine being approximately 50 times as potent as TEA and 1,000 times as potent as choline. When the accumulation of TEA, instead of NMN, was measured (22), tolazoline was the most potent inhibitor, NMN was considerably less potent while guanidine and methylguanidine had approximately the same low degree of potency. Darstine and choline were not used as inhibitors in this instance.

In another study with chickens, performed under conditions identical with

those described in the second paragraph of this section (II, 5), the inhibitor potency of three homologous series of organic bases for NMN transport was studied (34, 101). The three series consisted of tetraalkylammonium compounds, straight-chain aliphatic amines and 1-alkyl-1-methylpiperidinium compounds, Darstine being a member of the last. Many of these compounds were not studied with respect to whether they themselves were transported. In all three series, ability to inhibit NMN transport increased progressively as the length of alkyl substituents on the nitrogen was increased from methyl or ethyl through heptyl or octyl. With the tetraalkylammonium compound and the amines this was true whether the increase in chain length occurred on one or on all alkyl substituents. For example, TEA was four times as potent as trimethylethylammonium in inhibiting NMN transport while TBA and trimethyloctylammonium were 7½ to 15 times as potent as TEA. Hydroxylation of the ethyl group of trimethylethylammonium, which yields choline, decreased inhibitory potency for NMN transport as did hydroxylation of the terminal carbon of the butyl radical in 1-butyl-1-methylpiperidinium. In an unpublished study in Farah's laboratory, the ability of various aliphatic amines and tetraalkylammonium compounds to depress the S/M ratio of NMN in renal slices has yielded structure-activity correlations strikingly similar to those just described for the chicken.

If the relative ability of these compounds to inhibit NMN transport reflects their affinity for a transport system for strong organic bases, the structure-activity relationships described must have some significance. All these inhibitors are strong bases with pKa values of at least ten and in most cases higher; the pKa's of the members of each series differ only slightly from one another; *e.g.*, the pKa's of the aliphatic amines studied are all between 10 and 11, hence all exist virtually completely as cations at body pH. Therefore, the wide range of difference in ability to inhibit NMN transport between the lower and higher homologues in each series must be due to factors other than degree of ionization. The cationic nitrogen in these compounds is a hydrophilic group. The effect of an increase in the length of alkyl substituents would be to increase and that of hydroxylation to decrease the lipid solubility of the substituent. Therefore, affinity for transport may require a lipid-soluble or organophilic group in addition to the hydrophilic nitrogen. If so, the cellular membrane would be the most likely place where such a structural feature would be important. Another interpretation might be that the increase in molecular size increases the ability of the compound to offer steric hindrance and thus to inhibit the transport of another base. It is somewhat difficult to see how hydroxylation could have an attenuating effect under these circumstances, unless it exerts an effect on the shape of the molecule due to the attraction between the OH group and the positively charged nitrogen. The resulting neutralization of the charge on the positive nitrogen might also be involved. Such an attenuating effect of hydroxylation has received considerable attention with respect to compounds affecting neural transmission (74). Finally, it is possible that an increase in the length of the alkyl radicals in organic bases increases their ability to bind to a protein which serves as an intracellular "car-

rier" or receptor in the tubular transport system for such bases. Such a structural change is known to increase the binding of a number of acidic substances to serum albumin (33).

The studies described in this section have dealt with the comparative ability of various bases to inhibit NMN transport and with the relationship of chemical structure to this phenomenon. In view of the wide differences in potency of the various bases studied and in view of certain observations made with the organic acids, an assessment of the actual transportability of the bases themselves, in terms of Tm, and its relation to chemical structure, would be highly interesting. Höber (36) has pointed out that sulfonated dyes which are transported have a bipolar structure, the molecule having a hydrophilic and an organophilic end. Taggart (94) has called attention to similar features among a wide variety of compounds transported by the renal tubular excretory system for organic acids. Sperber (91, 92) found that sulfonated dyes which were the most potent as inhibitors of phenol red transport were themselves turned over slowly in the transport system for these dyes in the chicken kidney, as indicated by low Tm values. Unfortunately, adequate information concerning the Tm's of the strong organic bases studied is not available at this time and it is highly likely that the toxicity of some of them would preclude studies in this direction.

6. The effect of metabolic intermediates and inhibitors of cellular metabolism on the tubular transport of strong organic bases. Extensive sudies have been performed along these lines in the case of the organic acids, PAH and phenol red (5, 6, 15, 30, 56, 57, 82, 83, 93, 94, 95). The transport process for these substances in slices of mammalian kidney cortex is dependent upon aerobic metabolism since it is blocked by anaerobiosis, by inhibitors of the cytochrome system of electron transport, and by inhibitors of oxidative reactions of the tricarboxylic cycle. The oxidative energy is apparently made available to the transport system in the form of energy rich phosphate compounds (such as ATP) since it is inhibited by 2,4-dinitrophenol (DNP) and other "uncouplers" of oxidative phosphorylation (15, 95). As pointed out earlier, such data must be interpreted cautiously since inhibitors of such fundamental processes as oxidation and phosphorylation may affect cellular integrity on a broader basis; *e.g.*, through non-specific alterations in cellular permeability in the slice, so that the transport system of organic acids is only indirectly affected (94). In the case of DNP, however, it has been possible to reduce the Tm's of PAH, phenol red and Diodrast in intact dogs without simultaneously affecting the reabsorptive transport of glucose and glycine (57). Therefore, the effect of this inhibitor on the transport of organic acids appears to be a specific one and, on the basis of evidence from other clearance experiments, the same seems to be true for fluoroacetate and malonate (71) which inhibit oxidation of citrate and succinate, respectively, in the citric acid cycle. Furthermore, studies have been reported which propose a specific site of action of DNP and certain other inhibitors of PAH transport at specific postulated spatial steps in the transport system (32). The accumulation of PAH in slices of kidney cortex is stimulated markedly by acetate (30 to 100%) and to a lesser extent by pyruvate and lactate which are acetate precur-

sors (15). These stimulatory effects have also been shown in terms of an increase in the Tm of PAH in the intact dog, in the case of acetate and lactate (56). They also apply to phenol red (82, 83). In the case of acetate, particularly, the stimulation of PAH uptake in the slice is much greater in some instances than the simultaneous increase in oxygen consumption, a factor which leads to the logical belief that acetate has a specific role in the transport of organic acids (15, 93, 94). Succinate, α-ketoglutarate, fumarate, malate, the amino acids glycine, alanine and glutamate, and fatty acids of intermediate chain length decrease the accumulation of PAH by the slice, in spite of an increase in oxygen consumption (15, 94). This inhibitory action on PAH transport may involve the same mechanism as that which mediates the stimulatory effect of acetate, and in the case of the fatty acids of intermediate chain length and of alanine Schachter *et al.* (75) have presented evidence and arguments in this direction. In the case of succinate and fumarate, inhibition of PAH transport has also been demonstrated in the intact dog, without any depressant effect on the reabsorptive transport of glucose (56). The depressant effect of these and the other dicarboxylic acids mentioned above on PAH transport lacks an explanation at this writing. The summary of these extensive studies is purposely brief and is restricted to those aspects which have been applied in a more limited way to the transport of organic bases.

With the bases the most critical slice studies have been performed by Farah *et al.* who measured the effects of substrates and metabolic inhibitors on the simultaneous accumulation of PAH and TEA (21) and, in more limited studies, of PAH and NMN (20), in slices of dog kidney cortex. Similar studies have been performed with Darstine using slices of rabbit kidney cortex but direct and simultaneous comparisons with PAH in the same slices were apparently not performed (44). Thus, the results of the two studies are not directly comparable. On the other hand, the similarity of experimental conditions in the Darstine study to those used by Cross and Taggart in PAH studies (15) makes comparisons in this direction possible. Also the results obtained by Farah *et al.* (20, 21) in dog kidney slices in the case of PAH are in essential agreement with those of Cross and Taggart (15) who used slices of rabbit kidney cortex.

Table 2 represents a brief and rather gross summary of the most important results obtained. Except for the fact that lactate produced an unexplainable depression of the uptake of Darstine by the slice, acetate and its precursors stimulated the uptake of organic bases. However, the stimulation of PAH uptake was much greater. The lack of effect of succinate, α-ketoglutarate and octanoate on the uptake of TEA is in sharp contrast to the depressant effect of these substrates on the uptake of PAH. α-Ketoglutarate likewise had no effect on the transport of NMN, though both succinate and α-ketoglutarate appeared to depress the transport of Darstine. The metabolic inhibitors listed below these substrates in table 2 in general produced a depression of the uptake of either TEA or Darstine or both.

Some of the findings just presented have been applied to clearance studies in dogs (71), most of which involved simultaneous measurements of the Tm's of

TABLE 2

The effect of certain metabolic substrates and inhibitors on the accumulation of PAH and organic bases in slices of mammalian kidney cortex

(+ = stimulation; − = inhibition; 0 = no effect)

Substrate or Inhibitor	PAH (dog)	TEA (dog)	NMN (dog)	Darstine (rabbit)
acetate	+	+	+	+
pyruvate	+	+	+	+
lactate	+	+		−
succinate	−	0		−
α-ketoglutarate	−	0	0	−
octanoate	−	0		
anoxia	−	−		
cyanide	−	−		
antimycin A	−	−		−
malonate	−	0	0	
dehydroacetic acid	−	0		−
fluoroacetate	−	−	−	−
fluorobutyrate	−			−
iodoacetate	−	−		
arsenite	−			−
azide	−	−		
2,4-dinitrophenol (DNP)	−	−		−
phloridzin	−	0		

PAH and TEA. Acetate and lactate augmented the Tm of PAH markedly but did not alter the Tm of TEA, measured simultaneously. DNP decreased the Tm's of both PAH and TEA with a consistently greater effect on the former. Fluoroacetate and malonate reduced the Tm of PAH but did not alter that of TEA. In another study, dehydroacetic acid, an inhibitor of succinate metabolism, decreased the transport of NMN (83).

The aggregate of these findings is difficult to interpret with respect to the metabolic requirements of the tubular transport system for organic bases, particularly because of certain inconsistencies in the findings which may be based on species difference and differences in the exact experimental conditions employed (*e.g.*, concentrations of inhibitors). Earlier conclusions that acetate plays a specific role in the transport of PAH and other organic acids is unchallenged by the findings presented, while the less marked stimulation of uptake of base by acetate suggests that this effect is due largely to the increase in the metabolism of the slice. Unfortunately, oxygen consumption was not measured in the slice studies with TEA and Darstine. However, existing knowledge of the magnitude of effect of acetate and its precursors on the respiration of kidney slices supports this conclusion. Likewise, and despite the observed depressant effect of succinate and α-ketoglutarate on the uptake of Darstine, their effect and that of octanoate on PAH transport would seem to be a specific one which does not apply to the transport of organic bases. An essential and specific role of oxidative phosphorylation in the transport of organic bases, as well as that of organic acids, is suggested

by the fact that DNP depressed the simultaneous transport of TEA and PAH not only in the slice (21) but also in the dog (71), and by the fact that the doses of DNP used are known to have no effect on the reabsorptive transport of glucose and glycine (57). The action of the other metabolic inhibitors on the transport of all of these substances in the slice indicates that oxidative metabolism is essential to transport. However, whether the metabolic inhibitors, in the case of the slice, inhibited the transport systems directly or produced broader effects on cellular structure and permeability remains open to question. Since some of the inhibitors used in the slice studies have more than one site of action (*e.g.*, azide and phloridzin) the particular metabolic steps which might be essential to transport cannot be accurately identified (48). The finding that malonate and fluoroacetate depressed PAH transport *in vivo* but not that of TEA would suggest that the intactness of the citric acid cycle is more crucial for PAH transport than for that of organic bases. The possibilities have been suggested that the transport of TEA derives its energy principally from an oxidative mechanism not dependent on the citric acid cycle or that the more consistent inhibition of PAH transport by inhibitors of oxidation is due to the accumulation of intermediary metabolites of the cycle which inhibit PAH transport but not that of TEA (21). Obviously, the nature of the role of oxidation and oxidative phosphorylation in the transport of organic bases and its specificity cannot be accurately assessed at this time. With respect to oxidative phosphorylation, it might be of interest to study the transport of PAH and organic bases in slices under anaerobic conditions in the presence of a system which regenerates ATP, though the inability of added ATP to stimulate PAH uptake (15), possibly indicative of its limited ability to penetrate the cells of the slice, might well lead to inconclusive results in this situation as well.

A specific stimulation of the transport of strong organic bases by a metabolic substrate, analogous to the stimulation of PAH and phenol red transport by acetate, has not been reported to date. However, it has been shown that a number of alcohols, including ethyl, n-propyl and n-butyl alcohols and propylene glycol stimulate the accumulation of TEA in slices of dog kidney cortex (19). The accumulation of PAH was not significantly affected. In the intact dog neither the Tm of TEA nor that of PAH was altered by n-propyl or n-butyl alcohol but infusion loads of these alcohols were severely limited by their hypotensive action. The effect of ethyl alcohol in the dog was not reported. The stimulating effect of alcohol in slices was abolished by disulfiram. The interpretation of these findings is obscure at this time and for this reason a report of them may not even belong in this section of this review. Among a number of possibilities is that the alcohols may facilitate the passage of TEA across the cell membrane.

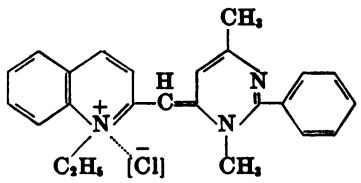
7. Inhibition of the tubular transport of strong organic bases by other bases which are "refractory" to transport. The tubular excretion of organic acids such as PAH, phenol red and penicillin can be inhibited competitively by the acid probenecid (5). However, the rate of renal clearance of probenecid in the dog is only a small fraction of glomerular filtration rate, indicating marked tubular reabsorptive rather than excretory transport of the inhibitor (8). Also, when probenecid is

infused into the renal portal circulation of one kidney of the chicken, only minute traces of it appear in the urine of either kidney even with large infusion loads (103). A logical interpretation of these findings is that probenecid has a high affinity for some component of the transport system for organic acids and yet is refractory to transport by the system (5). This situation has been appropriately compared to the competitive inhibition of succinate oxidation by malonate. Certain acidic sulphonphthalein dyes which are excreted by the renal tubules of the chicken kidney to only a slight extent, are potent inhibitors of the transport of phenol red (91). Such inhibitory dyes are stored in the renal tubular cells and a delay in their tubular transport is demonstrable. Some of the observations described below bear a striking resemblance to this phenomenon.

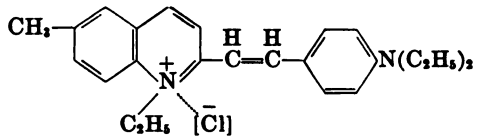
The tubular excretion of strong organic bases can also be inhibited by certain compounds, basic in nature, the net excretion of which is of a very low order of magnitude under the experimental conditions employed. Inhibitory behavior of this type has been demonstrated for basic cyanine dyes in the dog (63, 72), chicken (96, 98) and renal slice (20) and for bisquaternary ammonium compounds (96, 98), mecamylamine, quinine and quinacrine (96, 99, 100) in the chicken.

The basic cyanine dye #863 (1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride) has been shown to be a potent inhibitor of the tubular transport of NMN and TEA. Outstanding characteristics of the inhibition were its prolonged duration in the dog, its gradual and progressive development in the chicken at slow rates of infusion, the marked accumulation and firm binding of the dye in the renal tubular cells and the extremely slow rate of its own net renal excretion.

In dogs, single i.v. dose of only 1.25 μmol (0.5 mg) of cyanine #863 per kg reduced the high rate of renal clearance of NMN to GFR within ten minutes (63, 72). Following its injection, the dye accumulated very rapidly and in high concentrations in renal tubular cells, its accurately measurable concentration in the kidney being many times greater than that in any other tissue. Five μmol of cyanine per kg inhibited NMN transport specifically and completely for many hours. Reversal of the inhibition, slightly in evidence in 24 hours and almost complete in 96 hours, paralleled the slow rate of disappearance of the dye from the kidney. The rapid onset of the inhibition of NMN transport by cyanine resembled that seen with a rapidly transportable competing base such as Darstine but the two types of inhibition differed markedly in the speed of reversal which was immediate with the latter (40). Despite its high concentration in tubular cells, the dye appeared in the urine only in trace amounts.



1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride (#863)



N-(2-(*p*-diethylaminostyryl)-6-methylquinoline) ethochloride (#350)

A number of other cyanine dyes which accumulate in renal tissue also inhibited NMN transport (72). A styrylquinoline dye (#350; N-(2-(*p*-diethylaminostyryl)-6-methylquinoline) ethochloride) was only slightly less potent as an inhibitor than cyanine #863. Furthermore, #350 was not visually discernible in the urine even though its red color was apparent in aqueous solution in a concentration of only 1 μg/ml. Cyanine #863 also inhibited the tubular transport of TEA completely in a dose as low as 2.5 μmol/kg. The transport of Darstine was only partially blocked by a dose of 10 μmol/kg (40), a finding consistent with the fact that Darstine has a high affinity for the transport system (see above). Doses of 20 or more μmol of cyanine per kg produced renal tubular injury with a non-specific depression of overall renal function (63, 65).

When NMN and another, rapidly transportable, strong organic base (*e.g.*, Darstine) were infused simultaneously into the renal portal circulation of one kidney of the chicken, the inhibition of NMN transport reached a maximum within ten minutes and was reversed immediately upon cessation of infusion of the inhibitor (101). When cyanine #863 was infused into the portal circulation with NMN, the pattern of inhibition of NMN transport differed both from that seen with the cyanine in the dog and from that seen in the chicken with a rapidly transportable competing base (96, 98). When solutions dilute with respect to cyanine were infused at a slow rate, inhibition of NMN transport developed slowly and progressively. Complete inhibition was achieved with a wide range of concentrations of cyanine in the infusion, the time required being proportional to the concentration and being very short with high concentrations. Such a pattern of inhibition is consistent with the concept that the dye accumulates in tubular cells, with relatively firm binding to transport receptors (and possibly to other sites), rather than being rapidly transported into the lumen. The reversal of the inhibition with all infusions required approximately 40 to 60 minutes and was therefore much more rapid than that seen with the cyanine in the dog but significantly slower than that seen with a transportable competing base in the chicken.Kidney tissue removed from chickens after infusion of the cyanine contained a very high concentration of dye, the concentration being many times greater in the kidney on the injected side as compared to the opposite side. However, and in contrast to the dog, reversal of the NMN transport in the chicken was complete at a time when the dye content of the kidney was still very high. As in the dog, the renal excretion of the cyanine in the chicken was of a very low order of magnitude. ATEF values were initially very low and increased progressively, but exceeded 0.1 (10%) to a slight extent only upon prolonged infusion.

Inhibition of the accumulation of NMN in renal slices by cyanine #863 has recently been clearly demonstrated (20). This observation is made even more

important by the finding that, when the concentration of cyanine in the medium was relatively low, the inhibition could be reversed by increasing the concentration of NMN; *i.e.*, the inhibition was apparently a competitive one. When the concentration of cyanine was increased, this phenomenon of reversal was no longer demonstrable. These findings are compatible with the observation that NMN transport in dogs is inhibited by small doses of cyanine which do not affect other renal functions, but that much larger doses of the dye produce tubular injury, and that high concentrations inhibit the energy metabolism of renal tissue *in vitro* (2, 64, 65).

The dye itself has been shown to accumulate rapidly and in high concentrations in slices of mammalian renal cortex and to be firmly bound. The binding process conformed to an adsorption isotherm over a wide range of concentrations and was only partially depressed by anoxia and metabolic inhibitors. Microscopic examination of the slices revealed that the dye was present in the tubular cells rather than in the lumen. All areas of the nephron were stained though more dye was seen in the cells of the proximal convoluted segment. The same was true in slices of kidney derived from animals which had been given the dye intravenously (62). When homogenates made from renal cortical tissue of rabbits given the dye were subjected to centrifugal fractionation the greater part (80%) of the dye appeared in the mitochondrial fraction (72). While this may indicate that the dye was bound to a mitochondrial component essential to the transport of organic bases, cautious interpretation is necessary since redistribution may have occurred upon homogenization. A similar affinity of the dye for mitochondria was demonstrated when it was added to homogenates *in vitro*. Precise histological studies, designed to define the exact site of localization of the dye in intact cells of kidney cortex, have not been made to date.

Some of the observations described above require repetition in order to present a concise working hypothesis for purposes of further discussion. It is visualized that the receptor sites which bound the basic cyanine dye #863 so firmly within renal tubular cells consist, at least in part, of sites which play a role in the renal tubular excretion of rapidly transportable organic bases. The firm binding of the cyanine would make it relatively or completely refractory to transport and would account for the fact that it was excreted only in trace amounts. The binding of the rapidly transportable bases to the same receptors would be essential for their transport but would necessarily be transient and readily dissociable. Such a situation would permit the cyanine to serve as a potent competitive inhibitor of the transport of strong organic bases.

While the renal excretion of the cyanine in both dogs and chickens was of a very low order of magnitude, it should be pointed out that a tubular excretion of the dye may still have occurred at a slow and undetectable rate. Since it has an affinity for all renal tubular cells, the dye may have been excreted slowly via tubular transport in the upper part of the nephron and may subsequently have been rebound by tubular cells at a lower level. The dye has been shown to be excreted by the aglomerular fish, *Lophius americanus*, though the rate of this excretion was not reported (29). The fact that ATEF values for the cyanine in

the chicken increased progressively and exceeded 0.1 (10%) to a slight degree with prolonged infusion may reflect the fact that it diffused into the lumen from a progressively increasing concentration which was bound within the tubular cells. While some may argue that this does not represent transport in the true sense, recent studies with transportable organic acids suggest that a process of intracellular accumulation may also be involved in the transport of these compounds, even though such transport occurs at a rapid rate (30, 31, 32).

It is tempting to postulate that isolation and characterization of a component of renal tissue which binds the dye would reveal the nature of a receptor, "carrier" substance or enzyme in renal tubular cells, or on their surface, which participates in the rapid tubular transport of strong organic bases. The pattern of inhibition of NMN transport in the dog by cyanine supports such a possibility since the disappearance of the dye from the kidney and the regeneration of ability to transport NMN were parallel phenomena. However, this is not true in the chicken where regeneration of ability to transport NMN after cyanine infusion proceeded at a faster rate than did the disappearance of the dye from the kidney. Therefore, in this species, it would appear that a large portion of the dye in the tubular cells was bound to components which did not participate directly in base transport. Perhaps the same is true in the dog, and the prolonged inhibition of NMN transport in this species may have been due to the greater susceptibility of the transport system to inhibition by small amounts of dye slowly liberated from non-specific binding sites in the tubular cells. The differing relationships between NMN transport and the cyanine in the two species could also be due to the fact that in chickens the dye was infused into the renal portal circulation and therefore was presented to the tubular cells entirely from the interstitial side of the cells, while in the dog, where the dye was injected into the systemic circulation, both the interstitial and luminal side of the cells were exposed to it, since its incomplete binding to plasma protein would permit its appearance in the glomerular filtrate. In any case, even in the chicken, the reversibility of the inhibition occurred more slowly than it did in the case of inhibitors which were themselves rapidly transported. Therefore, even in this species the relatively firm binding of the cyanine to some transport receptor in tubular cells, as compared to the readily dissociable binding of rapidly transported bases to such a receptor, can be postulated, with the qualification that binding to receptors not concerned with transport of organic bases may also occur.

It is interesting to speculate upon the basis for such a firm binding of the cyanines. Their several nitrogens, widely separated by a conjugated carbon chain, may represent reactive cationic sites which can bind to the anionic sites of a cellular receptor. Since most of the readily transportable bases have only one nitrogen, one could visualize their rapid transport as involving transient binding by one-point attachment to the same receptor. The multiple-point attachment of the cyanines could be firmer and more slowly dissociable, with resulting inhibitory behavior and relative refractoriness to transport.

Though the cyanine may also be bound, in tubular cells, to receptors not concerned with organic base transport, unpublished studies by Dr. C. G. Huggins

in this laboratory, concerned with cyanine-binding components of homogenized rabbit kidney, are of interest. Among these components, a phospholipid fraction and particularly a cephalin fraction were outstanding. *In vitro*, this fraction bound not only the cyanine but also the transportable bases. Since the components of the cephalin fraction (phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl inositol) have a net negative charge due to their phosphate groups, they might be able to serve as anionic binding sites for cationic cyanines and transportable bases in the transport of the latter. Lecithin (phosphatidyl choline), which has no net negative charge, did not bind the transportable bases *in vitro* and bound the cyanine to only a slight extent. The transportable bases demonstrated competition for binding to the cephalin fraction *in vitro*. The binding of organic amines and inorganic cations to phospholipids has been reported in the past (11, 24, 45, 59, 85) and phosphatidyl serine has been proposed as a transport mediator for monovalent inorganic cations in erythrocytes (42). Woolley (106) has reported studies with a fraction derived from animal tissues known to respond to acetylcholine, which he proposes as an acetylcholine receptor, and has found its chemical behavior to resemble that of phosphatidies. Recently active choline transport has been proposed as a step in acetylcholine-mediated neurohumoral transmission (49, 50, see below) and nervous tissue is, of course, an excellent source of phospholipids. Since the cell membrane is also lipid in character, a role of phospholipids in the transport of organic bases, in renal tubular cells and at other sites, is one attractive possibility though certainly not to the point of excluding many others.

In addition to possessing positively charged nitrogen atoms, the cyanine molecule is one of large size. This in itself might sterically hinder its tubular excretion and hence the simultaneous tubular excretion of rapidly-transportable bases which are of smaller molecular size. Also, the conjugated system in the cyanine molecule may result in a flat configuration which would again introduce steric factors, *e.g.*, at the cell surface, leading to its slow turnover and inhibitory behavior in the transport system. These factors again imply combination with a receptor substance.

In the case of the bisquaternary ammonium compounds which produce ganglionic and neuromuscular blockade, the critical distance between the two cationic heads implies to some investigators a two-point attachment of the compound to anionic receptor sites which normally combine with acetylcholine, thus blocking the action of the neurohumor. Reasoning by analogy, it seemed possible that symmetrical bisquaternary compounds, like the cyanine, might block the tubular transport of organic bases. Whether on the basis of such a mechanism or not, this is the case (96, 97, 98). Hexamethonium, which is itself transported by the renal tubules at a very slow rate in the chicken, did not block the transport of NMN in this species in the doses used. It may be recalled that the rapidly transportable compound TMA was a poor inhibitor of NMN transport, but that compounds in which one or more of the methyl groups of TMA were replaced with larger radicals demonstrated inhibitory properties (34). This principle was therefore applied to the bisquaternaries with similar results. Compounds in which

the methylene bridge was still 6 carbons in length and which are potent neuropharmacological agents (96, 97, 98), but in which the alkyl substituents on each quaternary nitrogen were dimethylethyl, diethylmethyl, dimethylpropyl, dimethylbutyl and dimethylisobutyl, *did* inhibit NMN transport. The pattern of inhibition produced by dilute infusions of these compounds was gradual and progressive in onset, as in the case of the cyanine, but reversal was immediate. Complete inhibition could be achieved, though infusion loads required for this on a molar basis were much larger than those of cyanine #863. The pattern of distribution of these bisquaternary compounds (including hexamethonium) in rats resembled that of the cyanine, in that they showed the same highly selective affinity for renal tissue. The rates of excretion of the inhibitory bisquaternary compounds in the chicken also resembled that of the cyanine, ATEF values being below 0.1 (10%). One chicken was sacrificed immediately following the infusion of one of the inhibitory bisquaternaries. As with cyanine, the concentration of the compound in the kidney on the injected side was found to be extremely high and much greater than that in the kidney on the control side. In a similar experiment involving the rapidly transportable base TEA, the concentration of infused compound in the two kidneys was of a very low order of magnitude, with a smaller differential.

This action of the bisquaternary agents encourages further consideration of the possibility that neurohumoral transmission by the basic neurohumoral mediators and the tubular transport of strong organic bases may have certain features in common and that the receptors involved might be chemically related. In this regard the pharmacological action of the hemicholiniums and the mechanism of action proposed for them by MacIntosh *et al.* (49, 50) are of particular interest. The hemicholiniums have been shown to produce respiratory paralysis of central origin which is delayed in onset and is antagonized by either eserine or choline, suggesting that paralysis is based on interference with a cholinergic mechanism (76). Under certain experimental conditions, transmission over cholinergic autonomic pathways was blocked by one of these compounds (HC-3) when the rate of stimulation was of high frequency. The block was characterized by a delayed onset and was reversed by injection of choline. Also, HC-3 inhibited the synthesis of acetylcholine in a preparation of minced mouse brain *in vitro*, the inhibition being reversed by addition of choline. Since the drug did not inhibit acetylcholine synthesis in a cell-free system, MacIntosh *et al.* postulated that HC-3 inhibited acetylcholine synthesis, not by inhibiting choline acetylase or coenzyme A, but rather by inhibiting, competitively, the transport of choline to intraneuronal sites of acetylation by a specific carrier system (49, 50). As indirect support for this hypothesis it was shown that HC-3 effectively and reversibly inhibited the transport of choline by the renal tubules of the chicken without affecting the simultaneous transport of phenol red (50). Unpublished experiments in Farah's laboratory have shown that HC-3 is a potent inhibitor of NMN transport in renal slices. These studies also showed that eserine and neostigmine were potent inhibitors of NMN transport in the slice. However, since DFP was inactive it is doubtful that cholinesterase is the cellular mediator

in tubular transport of organic bases. It is in fact possible that physostigmine and prostigmine are themselves transportable bases or that they inhibit transport by mechanisms similar to those to be discussed in the next section.

Since histamine is excreted by the renal tubular transport system for strong organic bases, a relationship between the cellular receptors of this system and receptors for histamine in other cells also suggests itself. The recent findings that antihistaminics can serve as potent inhibitors of the tubular transport of NMN in the chicken and that two antihistaminics, diphrenhydramine (Benadryl) and antazoline (Anistine), inhibited the tubular transport of histamine (104) at first seem to support this suggestion. However it is opposed by the finding that the relative potencies of antihistaminics as inhibitors of the tubular transport of NMN and histamine differed markedly from their reported relative potencies as pharmacological antagonists of histamine.

Mecamylamine, quinine and quinacrine (Atabrine) produced a rapid, complete and readily reversible inhibition of the transport of NMN when infused simultaneously with it into the renal portal circulation of the chicken (96, 99, 100). ATEF values for mecamylamine and quinine were below 0.1, suggesting little or no transport of the inhibitors themselves in this species. The significance of these observations is not clear but, since the tubular transport of these amines in the dog can be altered markedly by altering urine pH, a possible relationship between active transport of strong organic bases and renal tubular excretion based on "non-ionic diffusion" (55) may exist. Therefore, these observations are discussed in the next section.

III. TUBULAR TRANSPORT OF WEAK ORGANIC BASES BY PASSIVE "NON-IONIC DIFFUSION"

The rate of renal excretion of a number of organic bases has been shown to vary inversely with the pH of the urine in the dog or in man (18, 35, 37, 55, 61). With some the renal clearance rate exceeded GFR when the urine was rendered strongly acid by ammonium chloride, while urinary alkalinization with sodium bicarbonate or acetazoleamide (Diamox) reduced the clearance to lower values consistent with tubular reabsorption. The mechanisms invoked to explain this pattern of excretory behavior have been very clearly formulated by Orloff and Berliner (61) and have been reviewed and evaluated recently by Milne *et al.* (55). Therefore, details of mechanism and historical background will not be presented here. Rather, the concept will be presented briefly to acknowledge its importance, to draw contrasts between it and the active tubular excretory transport of strong organic bases and to raise the question as to whether the two phenomena are actually related.

The process of tubular transport by "non-ionic diffusion" is explained on the basis that certain weak organic bases exist at the pH of blood, body fluids and urine as an equilibrium mixture of the unionized and ionized forms, and that the cell membrane is much more permeable to the unionized or lipid-soluble member of the pair than to the ionized, positively charged, lipid-insoluble member. When the pH of the tubular fluid is different from that of plasma in the peritubular

capillaries, the ratio of unionized to ionized form will be different in the two compartments. Thus, if one assumes for the moment that the concentration of total base on the two sides of the tubular cell is approximately the same, when the urine is acid the concentration of the unionized form will be greater on the interstitial side of the cell where the pH is above 7 than in the tubular lumen where the pH is lower. The resulting concentration gradient for the unionized form will therefore permit its passive diffusion from the peritubular blood across the tubular cell into the urine. Conversely, when the fluid in the lumen is rendered more alkaline than that of the plasma, there will be more of the unionized form in the lumen than in the peritubular blood and the resulting concentration gradient will favor passive reabsorption. This explanation of course assumes a marked degree of preferential permeability of tubular cells for the unionized form and the rapid attainment of equilibrium between blood and tubular fluid. In actual fact, the concentration of total base on the two sides of the cell would not have to be the same, as long as the pH difference were sufficiently great to create a concentration gradient for the unionized form in the appropriate direction.

Among weak organic bases which have demonstrated this excretory pattern are quinacrine (pKa 7.7), and procaine (pKa 8.95). The clearance of quinine (pKa 8.3) likewise varied inversely with the pH of the urine but did not exceed the filtration rate even at the extreme acidic end of the urinary pH range. It is obvious from examining the pKa values of these compounds that their degree of ionization can be altered considerably over the pH range (5 to 8) achievable in urine by administration of acid or alkali.

It is implicit in such a passive mechanism of tubular reabsorption and excretion that substances to which it applies should not require metabolic energy for their tubular excretion (or reabsorption). Furthermore, they should not exhibit competition for migration across tubular cells and should not exhibit tubular excretion and reabsorption maxima (*i.e.*, Tm's), since no rate limiting transport mediator is involved. Information along these lines is not available at this writing despite its importance to the concept presented.

Mecamylamine (3-methylaminoisocamphane), a secondary amine, occupies an interesting position in this situation. Baer *et al.* (1) found its rate of renal clearance in the dog to be far below GFR when the urine was alkaline but far above GFR when the urine was rendered acid. Mecamylamine is a relatively strong base with a pKa of approximately 11.4, so that very little of it can be in the unionized form at any one time at the pH of the blood and over the pH range achievable in urine. At a pH of 7, 1 molecule in 25,000 is in the form of the free base and an increase to only 10 molecules in 25,000 is produced by increasing the pH to 8; yet the net clearance of mecamylamine changed from a value below GFR to one exceeding it over this urinary pH range. Thus, while the *relative* decrease in the unionized form which can be produced in the urine by acidification and the relative increase which can be produced by alkalinization is greater for mecamylamine than for weaker bases, the actual number of molecules of base available for movement across the cells at any instant is small. For this reason, Baer *et al.* (1) left open their interpretation concerning the

mechanism of movement of mecamylamine across tubular cells and suggested that some mechanism other than "non-ionic diffusion" may explain its clearance behavior in relation to urinary pH. For example, they suggested that the ionized form may participate in cation or hydrogen exchange mechanisms or that active tubular reabsorption and excretion may occur at the same or separate sites through pH-dependent mechanisms. Others disagree with this interpretation. For example, Milne *et al.* (55) placed mecamylamine in the same category as the other bases transported by "non-ionic diffusion" on the assumption that the ratio of permeability of the unionized form of mecamylamine to that of the ionized form may be higher in the case of this base than in the case of bases with lower pKa values. Under such circumstances, rapid diffusion of the unionized form across the tubular cells could still account for the excretion pattern observed, and for the magnitude of tubular excretion and reabsorption of mecamylamine, despite the small number of molecules of the unionized form involved at any single instant.

While available evidence indicates that the organic bases discussed in the previous section of this review are excreted by the renal tubules by active transport, studies concerning the effect of urinary acidification and alkalinization on their rate of renal excretion in the dog have not been reported. It is difficult to present a critical discussion of the relationship between their mechanism of tubular excretion and the pH-dependent mechanism of tubular excretion and reabsorption proposed for the bases under discussion in this section, since similar studies have not been applied extensively to both groups. However, in view of the excretory behavior of the relatively strong base mecamylamine and its interpretation as an entirely passive, pH-dependent process, a concept of obligatory relationship between strong basicity and active tubular transport in an excretory direction only, which could be implied from the emphasis upon *strong* bases in the previous section of this review, should not be adopted at this time. This is also true because the active tubular excretion of a very weak base in aglomerular fish has been demonstrated (29).

Recent studies from this laboratory (96, 99, 100) could be interpreted as indirect evidence for a relationship between the active tubular transport of organic bases discussed in the previous section and the pH-dependent movement of quinacrine, procaine, quinine and mecamylamine across renal tubular cells. These studies have shown that quinine, quinacrine and mecamylamine, infused with NMN and PAH into the renal portal circulation of one kidney of the chicken in appropriate amounts, produced a rapid, complete, readily reversible inhibition of the transport of NMN, this pattern of inhibition being identical with that produced by Darstine and other rapidly transportable bases. Quinine was 2.5 to 5 times as potent an inhibitor as were quinacrine and mecamylamine, hence inhibitory potency did not correlate with pKa. Quinine also inhibited the tubular transport of C^{14}-labeled TEA while quinacrine and mecamylamine were not studied in this regard.

Surprisingly, the ATEF's of quinine and mecamylamine and the ratios of these to the ATEF of PAH, determined in the absence of NMN, were very low

(usually below 0.1) even though the urine of the chickens was acid (pH 5 to 6.5). This finding is difficult to explain. It could represent a very fundamental difference between the mechanisms of renal excretion of bases such as mecamylamine in the two species. However, the difference might also be explained on simple physical grounds. If the movement of mecamylamine from peritubular blood to tubular lumen *is* based on passive diffusion, the low ATEF's might be explained on the basis that the small volume of glomerular filtrate in proportion to renal blood flow in this species provides a poor physical system for a rapid diffusion process (see METHODOLOGY). A much steeper and unattainable gradient would then be required to promote substantial diffusion of the non-ionic form of the base in this species than in the dog.

Quinine, quinacrine and mecamylamine may act in this situation as competitive inhibitors of NMN and TEA transport while being themselves refractory to transport or they may act as non-competitive inhibitors by an unknown mechanism. In either case, the active transport of NMN and TEA could be completely unrelated to what is currently interpreted as a bidirectional, passive, pH-dependent movement of quinine, quinacrine, and mecamylamine across the tubular cells in the mammalian kidney. Since the excretion of mecamylamine in the chicken was of a low order of magnitude, despite the acidity of the urine, proof or disproof of such a relationship can be derived only by extending these studies to dogs. If inhibition of NMN transport by quinine, quinacrine and mecamylamine were demonstrable in this species and if the high rate of clearance of mecamylamine in dogs with low urine pH could be depressed by potent inhibitors of NMN transport such as cyanine #863 and Darstine, a relationship between what is presently regarded as two separate processes would seem highly likely. Such experiments, recently initiated in this laboratory, have also been suggested by Lotspeich (48) without knowledge of our findings.

A considerably earlier speculation of Jailer *et al.* (37), concerning the enhancing effect of orally administered ammonium chloride on the urinary excretion of quinacrine and several other basic antimalarials in man and the reduction of such excretion by sodium bicarbonate, is highly pertinent to the present discussion. Without presenting experimental evidence, they postulated a three-phase system to be operative in the renal excretion of these compounds, the first phase consisting of glomerular filtration of the unbound fraction of the drug and the second of active tubular excretion of the drug at the level of the proximal convoluted tubule. The third phase was proposed to be a passive reabsorption process involving the concentrated urine in the lumen of the distal tubular segment. Here alkalinization would provide a high ratio of the unionized, permeable form to the ionized, impermeable form of the drug and thus a favorable gradient for reabsorption of the former. Acidification would decrease the ratio of unionized to ionized form and would thus retard reabsorption. On the basis of such a mechanism, it would not be unreasonable to expect that quinacrine, mecamylamine and quinine could compete with NMN and TEA for active tubular transport at the level of the proximal convoluted tubule in the mammalian kidney and could still demonstrate renal clearance rates below GFR when the urine is alkaline.

Finally, it should be pointed out that the ability of urinary acidification to *decrease* the renal excretion of certain organic acids and of alkalinization to *increase* such excretion has also been explained as a passive "non-ionic diffusion" process, analogous to that described above for the bases which, of course, react to pH change in the opposite direction. Highly convincing experimental evidence that this process for acid excretion involves a three-phase system of filtration, active transport, and passive reabsorption similar to the above has recently been presented by Weiner *et al.* (103) in the case of salicylic acid, the active transport phase being susceptible to inhibition by PAH and probenecid, and the passive reabsorptive phase susceptible to enhancement by urinary acidification and to depression by urinary alkalinization and diuresis. Evidence for a similar multi-phase system of filtration, active tubular excretion and passive pH-dependent reabsorption in the mammalian kidney may also be obtainable for organic bases such as mecamylamine, quinacrine, quinine, *etc.* Until such evidence is obtained and in view of the unexplained low ATEF's of mecamylamine in chickens with acid urine, continued consideration must be given to the possibility that the movement of these bases across the renal tubules in mammals is passive and pH-dependent in both directions.

IV. TRIMETHYLAMINE OXIDE AND TRIMETHYLAMINE

Trimethylamine oxide (TMAO) is a weak base which is a normal constituent of the plasma in at least some species of fish. In the goose fish *Lophius americanus*, which has aglomerular nephrons, TMAO frequently contributes more than 50% of the total urinary NPN. Forster *et al.* (29) have inhibited rapid tubular excretion of TMAO in this species with i.m. injections of TEA and cyanine #863. The rapid tubular excretion of TEA also was demonstrated. Since cyanine #863 imparted a distinctly yellow color to the urine, it was concluded that the dye was actively transported. Also, the tubular excretion of TMAO was not inhibited by probenecid under conditions which markedly depressed the simultaneous tubular excretion of PAH. Attention should be called to the fact that TMAO is a weak base (pKa 4.5), since the active tubular transport of organic bases in the dog and the chicken has been demonstrated to date only for strong organic bases with high pKa values.

The kidney of the spiny dogfish, *Squalus acanthias*, is glomerular and in this species TMAO is very effectively reabsorbed from the glomerular filtrate, rather than being transported in an excretory direction (12, 13). This reabsorptive process was not inhibited by TEA which was itself transported in an *excretory* direction, nor by cyanine #863 (13, 29). The tubular reabsorption of TMAO in *Squalus* was inhibited by trimethylamine and by dimethylamine but not by monomethylamine, all of these amines being strong bases. This last observation is reminiscent of the fact that progressive alkyl substitution on the nitrogen of aliphatic amines increased their ability to inhibit NMN transport in the chicken, although, at the infusion loads used, higher homologues than the methylamines were required to inhibit NMN transport (34). The urine of *Squalus* contains a volatile amine which is not ammonia but is believed to be trimethylamine. When trimethylamine was administered subcutaneously to *Squalus* and the excretion

of the free amine was measured, the figures were consistent with a rapid rate of tubular excretion (13).

V. UREA, CREATINE AND CREATININE

These very weak bases are all excreted by the tubules of some vertebrate species but not others. Various aspects of their behavior necessitate their being discussed in a separate section. Extensive and detailed review of their excretion characteristics by Smith (84) makes it possible, for the sake of brevity, to confine the discussion to their relationship or lack of relationship to the tubular excretion of the bases described above, without complete reference to original work.

The renal clearance of urea in most mammals is less than GFR and its behavior indicates that reabsorption from glomerular filtrate is based on passive back diffusion. On the other hand, active reabsorption of urea has been reported in the case of dogfish and other elasmobranchs (28, 41). Marshall and Crane (53) showed that urea was excreted by the renal tubules of the frog where elevation of the plasma level of urea decreased its excretory efficiency, indicating saturation of an active transport process (52). Forster (26) demonstrated a Tm for urea in the bullfrog and showed that its tubular excretion was completely blocked by DNP and probenecid and partially by PAH. The tubular reabsorption of urea by the dogfish was not blocked by doses of probenecid which significantly reduced the tubular excretion of PAH (28). Thus, the tubular excretion of urea in the frog is apparently an active process mediated by the transport system for tubular excretion of organic acids such as PAH. Whether this tubular excretion of urea can also be inhibited by cyanine #863 and by rapidly transportable strong organic bases, *i.e.*, whether it can also proceed via the transport system for strong organic bases, is not known, but this seems unlikely.

Tubular excretion of creatinine has been reported for humans, anthropoid apes, rats, chickens and various species of fish, both glomerular and aglomerular. The average creatinine:inulin clearance ratio is relatively high in glomerular fish, *e.g.*, the dogfish *Squalus acanthias* (78), but low in chickens and mammals. An average figure of only 1.4 has been derived for man from a large number of studies (84). Because the figure is low and because other materials in plasma interfere with some methods of creatinine determination, the question as to whether creatinine is actually excreted by the tubules in man has been a controversial one. However, other evidence definitely supports the conclusion that it is. As in other species (the chicken and *Squalus acanthias*) (77, 78, 80), the creatinine: inulin clearance ratio in man could be depressed by increasing the plasma concentrations of creatinine and by the administration of phloridzin (79). Furthermore, the ratio in man was depressed by large doses of Diodrast and PAH (14) and by carinamide (10). Thus, it is concluded not only that active tubular excretion of creatinine can occur but also that the transport system involved is the same as that mediating the transport of organic acids such as PAH. The latter was supported by Fingl (23), who showed that the tubular excretion of creatinine in the rat could be depressed by PAH and probenecid. Creatinine is simply filtered without tubular excretion or reabsorption in a number of mammalian species including the dog. The fact that exogenous creatinine can be excreted by

the renal tubules of the chicken was demonstrated by an experimental procedure in which inulin and creatinine were injected into the systemic venous circulation and urine was collected together, rather than separately, from the two kidneys (80). Studies in which tubular excretion of creatinine was measured during its infusion into the renal portal circulation of one kidney of the chicken have not been reported. The finding that phloridzin blocked the tubular excretion of creatinine in various species is not of assistance in attempting to classify creatinine with respect to transport systems since phloridzin is apparently not highly specific in its inhibitory action (5). Since tubular excretion of creatinine is apparently mediated by the transport system for organic acids, it is doubtful that it could also be excreted by the transport system for organic bases. In this regard, exogenous creatinine did not inhibit the tubular excretion of TMAO in *Lophius* even though creatinine was itself excreted (29).

Creatine is reabsorbed from the glomerular filtrate in mammals, this reabsorptive process being susceptible to inhibition by glycine which is apparently reabsorbed by the same transport pathway (67). Creatine predominates over creatinine in the urine of birds, reptiles and certain fish (84). A high rate of tubular excretion of creatine by the glomerular fish *Squalus acanthias* has been demonstrated (66). Since the creatine:inulin clearance ratio could be decreased by increasing the plasma concentration of creatine and since a maximal tubular excretory capacity for creatine was demonstrated in this species, the tubular excretion of creatine was concluded to be an active transport process. This process was not susceptible to inhibition by phloridzin in *Squalus* but it could be inhibited in the teleost *Epinephalus morio* (29, 66). In *Lophius*, creatine along with TMAO constitutes the greater part of the total NPN. Creatine is therefore also rapidly excreted by the aglomerular nephrons of this species, but even large intramuscular doses of exogenous creatine did not depress excretion of TMAO (29). Furthermore, the transport of creatine was not inhibited by TEA or cyanine #863 in concentrations which effectively inhibited the tubular excretion of TMAO. Exogenous creatinine also did not inhibit the tubular excretion of creatine in *Lophius* nor did probenecid in concentrations which effectively depressed the transport of PAH. The negative results of these inhibitor studies preclude the classification of creatine, with respect to its tubular excretory characteristics, with any of the other compounds discussed in this review, at the present time.

VI. SUMMARY

A number of strong organic bases, including amines and quaternary ammonium compounds, can be excreted by the renal tubules in the dog and the chicken. The excretory process appears to involve active transport. The transport system is different in at least some of its components from that which can mediate the transport of organic acids such as PAH, phenol red and Diodrast. Available evidence indicates that the 14 strong organic bases listed use a single transport system and that they differ markedly in their affinity for the system as measured by their ability to inhibit the simultaneous tubular transport of N^1-methylnicotinamide (NMN). Inhibitory potency for NMN transport in homologous

series of compounds increased as the length of alkyl substituents was increased and decreased when these substituents were hydroxylated, indicating that not only the hydrophilic nitrogen but also an organophilic component of the molecule is involved in the transport process. The transport of strong organic bases was inhibited in renal slices by inhibitors of oxidation and oxidative phosphorylation. Whether this indicates a direct dependence of the transport system upon oxidative energy is not clear at this time. The transport system was inhibited by a number of substances which were themselves relatively refractory to transport under the experimental conditions employed. Some of these inhibitors contain several nitrogens. It is postulated that they bind relatively firmly, *e.g.*, by multiple-point attachment, to an anionic receptor in tubular cells, and that this same receptor mediates the rapid tubular transport of organic bases such as NMN and TEA by a mechanism involving transient, readily dissociable binding. The possible relationship of the receptors involved to receptors at other sites such as the nervous system is discussed.

The rate of renal excretion of a number of other organic bases, mostly weaker than the above, varies inversely with the pH of the urine in the dog or in man. When the urine is acid the rate of renal clearance of some of these bases exceeds glomerular filtration rate. It has been proposed that the tubular cells are permeable only to the non-ionic form of these bases and that the pH dependence of their movement across the tubular cells is based on the concentration gradient of the non-ionic form between peritubular blood and urine. Thus passive diffusion across the tubular cells in the direction of the lumen can occur when the urine is acid. The fact that such bases inhibited the tubular transport of strong organic bases in the chicken suggests the possibility that the two processes may actually be related, although the renal excretion of the inhibitors themselves in this species was minimal.

Trimethylamine oxide is a weak base but is actively excreted by the renal tubules of aglomerular fish, the transport mechanism being related to that which mediates tubular excretion of strong organic bases in mammals and birds. The reabsorption of trimethylamine oxide by the tubules of aglomerular fish was inhibited by trimethylamine.

The renal tubular excretion of urea and creatinine in certain species is apparently mediated by the transport system for organic acids, such as *p*-aminohippuric acid and phenol red, rather than that for strong organic bases.

The tubular excretion of creatine cannot be classified into either group at this time.

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