INVITED COMMENTARY

Transport of ^{99m}Tc-MAG3 via Rat Renal Organic Anion

The terms *molecular imaging* and *molecular biology* are being used increasingly in modern medicine. This reflects not so much a change in what we do as it does a refinement in our understanding and the conceptual approach to what we do. This advancement is not restricted to medicine but pervades our daily life. Our ability to achieve finer and finer detail is reflected in a wide range of devices, from the computers we use in our daily work, which have become smaller, faster, and more powerful, to those things that occupy our leisure time. Turning on a high-fidelity set at home provides us with increasingly accurate reproduction of sound as it was originally generated, through improving technology and our ability to reproduce smaller and smaller bits of the original.

The report by Shikano et al. (*1*) in this issue of *The Journal of Nuclear Medicine* describes a study using the organic anion transporter 1 (OAT1) in *Xenopus laevis* oocytes. This model of the isolated transporter made it possible for the investigators to study ^{99m}Tc-mercaptoacetylglycylglycylglycine (MAG3) transport by OAT1 and the in vitro inhibition of its uptake by *p*-aminohippuric acid (PAH), hippuran (OIH), probenecid, and other compounds. These data help us to understand in greater clarity how the kidney handles ^{99m}Tc-MAG3. It is possible to obtain similar information without isolation of the transporter, but the experiments are much more complicated and expensive since they usually have to be performed in vivo and the results are not as specific. The classic text by Homer Smith, *The Kidney: Structure and Function in Health and Disease* (*2*), reviews many experiments on intact organisms and humans. Although the words *transport*, *receptor*, and *anion* do not appear in the index, *hippuran* does. In a section on competition in tubular transport (page 202), Professor Smith reports on how competition between a variety of substances affects their net excretion. He notes that the authors of these studies interpreted their results in terms of "competition for available energy." Early on it was recognized that energy was expended to move PAH into the cell and then into the urine. The concept that there were specific molecules (transporters) to achieve these actions was an abstract thought at that time.

Throughout the subsequent years, numerous studies have been done on intact animals and sometimes on humans in which compounds have been infused simultaneously to determine their interaction. In some situations, the effect of an agent on renal function was measured using a standard of measurement such as PAH without any means of identifying whether the infused agent was affecting renal function per se or was affecting the transport of PAH. So, too, has it been difficult to differentiate the relative effects of renal transport and reabsorption and, except in a relatively crude manner, to identity the portion of the renal tubule involved in the excretion of a compound. This lack of refinement has sometimes fueled controversy over how a particular compound is handled by the kidney and, more important, over the significance of changes in its excretion. The isolation and measurement of transport of compounds and competition for transport by specific kidney transporters in in vitro models of the type used in this study serve several important functions. First, they help us to better understand how a specific compound is excreted and, therefore, what it is we are measuring when we use that compound in studying the kidney. Also, and perhaps equally or more important, by having the transporter available to us it is possible to prospectively construct compounds that are more ideally suited to transport by that molecule and, therefore, that can potentially provide us with more specific and useful information about the component of kidney function in question.

The story of how we arrived at the state of knowledge that made the subject of this commentary possible is an educational and instructive lesson.

Early studies as noted previously were done on intact organisms or in some cases on kidney slices or kidneys that had been excised from an animal and were perfused in vitro. In an article on renal tubular transport, Cross and Taggart (*3*) stated that "investigations on the biochemical mechanisms involved in active renal transport are difficult to approach with the various experimental techniques now available." The step forward at that time, which was reported in that study, was the use of rabbit kidney slices contained in a Warburg apparatus. The authors were able to show that slices of rabbit kidney cortex could accumulate PAH against a concentration gradient. They also showed the stimulatory effect of acetate on PAH transport.

Subsequently, innumerable studies of PAH transport were performed on a wide variety of biologic models. Moller and Sheikh (*4*) reviewed the

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progress as of 1983 in their article on renal organic anion transport. It is interesting that this extensive review included in its introduction a discussion of the medical and physiologic importance of renal organic anion systems. The authors noted that much of our knowledge on the secretory function of the kidney is based on its excretion of foreign organic anions. They also noted that "the primary function of the renal organic anion secretory system seems to be elimination of foreign compounds that are only catabolized to a limited extent and in high doses may be regarded as toxic to the body." Paradoxically, the authors noted that this system, which is thought to have evolved for protection, can also turn on itself as in the case of cephalosporidine. Nephrotoxicity is correlated with the intracellular accumulation of that compound in the kidney. In their section on medical importance, the authors made note of OIH scintigraphy, indicating that in studying patients with renal failure or after transplantation, it is assumed that intracellular accumulation of organic anions is dependent on optimal functioning of the kidney. That view has prevailed throughout the years among practitioners who use nuclear medicine to study the kidney, with a strong emphasis on OIH even in the face of most nephrologists' dependence on glomerular filtration as a marker for changes in renal function.

Numerous other reviews have appeared. In 1993, Pritchard and Miller (*5*) linked mechanisms mediating renal secretion of organic anions and cations and noted that many of the transport systems in the kidney are shared with the liver. This has been a repeated observation in nuclear medicine for most of the compounds used in studying renal function. It is well recognized that, in renal failure, hepatic uptake may lead to erroneous interpretation of many renal imaging studies. Although these studies led to an increasingly sophisticated knowledge of renal transport and of the factors that affect it, highly specific and detailed information still eluded us.

Contemporaneous studies continued to be relatively crude both in nephrology and in nuclear medicine. For instance, a study from my laboratory examined the biochemical and physiologic factors affecting dimercaptosuccinic acid (DMSA) uptake in the whole animal, which led to some conclusions about the probable mechanism of DMSA secretion that continue to be of controversy. Ongoing developments make clear that we will soon see this controversy resolved definitively (*6*).

At the time that nuclear medicine took a giant leap forward in the evaluation of kidney function with the introduction, by Fritzberg et al. (*7*), of ^{99m}Tc-MAG3 as a replacement for OIH, our interpretation was still based on macroscopic examination of the whole organism. They observed the high protein binding of ^{99m}Tc-MAG3. Effects such as protein binding on excretion of a compound are important to bear in mind because, even in the face of the remarkable advances noted below, they remain difficult to evaluate, except in the intact organism. Fritzberg et al. noted the "competition affinity of [^{99m}Tc]MAG_3 and OIH for the tubular transport system." They also noted hepatic accumulation, but the exact mechanism for excretion of ^{99m}Tc-MAG3 was beyond their resources.

A major change in that situation occurred with the April 1997 publication of Sekine et al. (*8*) that was directed at the expression, cloning, and characterization of a novel multispecific OAT. Again, they noted that the stimulus for isolation of this transporter was the important role of OATs in the excretion of foreign compounds. They reported the cloning and characterization of a novel multispecific OAT, OAT1. They isolated a rat kidney cDNA encoding a 551 residue protein. The transporter was expressed in *Xenopus laevis* oocytes for study in a living cell. They showed its ability to transport PAH. The transporter was shown to be nonspecific, acting on cyclic nucleotides, prostaglandin, uric acid, antibiotics, nonsteroidal antiinflammatory drugs, diuretics, antineoplastics, and uricosurics. It was shown to be expressed at the basolateral membrane of the proximal tubule. The authors stated, "isolation of OAT1 will facilitate elucidation of the molecular basis of drug kinetics and the development of new drugs lacking unwanted side effects." In another report (*9*), Sekine et al. noted the structural similarity to organic cation transporters. Interestingly, at meetings of the Society of Nuclear Medicine, papers suggesting the use of organic cations for studying renal function have occasionally been presented. The major organic anions include cyclic nucleotides, prostaglandin, urate, dicarboxylates, and a wide variety of other substances. OAT1 is present in kidney, liver, brain, and placenta. So, at that time, the PAH transporter had been identified and was named OAT1, suggesting that this transporter on the basolateral portion of the tubular membrane is responsible for the initial uptake of PAH into the cell. The transporter is sodium independent. Numerous other transporters have been described with similar nomenclature, and they are characterized by their preferential transporting of hydrophobic organic anions.

A few months later, Sweet et al. performed similar experiments with tritium-labeled PAH using OAT1 from the rat kidney. In the introduction of their report (*10*), the authors stated, "in contrast to the physiology of the organic anion transport system, precise information about the structural properties of the transport proteins that make up this system are not yet available. However, considerable progress has recently been made for a variety of other transport proteins through the application of expression cloning techniques leading to increased understanding of the regulation of their expression, identification of substrate binding sites and a much more complete appreciation of their mechanisms of action."

An apical membrane transporter now has also been identified (*11*), giving us a more complete "microspective" on PAH transport as it moves across the basolateral membrane into the cell and then out into the urine.

This study was accomplished by cloning the human transporter and transfecting it into cells for study. Numerous studies and reviews have subsequently appeared in the literature about this subject (*9,12–16*).

This remarkable progress is perhaps exemplified best in the titles of the various papers cited, in which words such as *molecular cloning*, *molecular physiology*, and *molecular characterization* are now commonplace. This discussion takes us full circle to the article that is the subject of this commentary. Although radiolabeled PAH has been the substrate of favor in almost all studies of the transporter, the current study looked at this transporter from a nuclear medicine perspective and showed that, indeed, the basolateral membrane transporter OAT1 is shared for PAH and for ^{99m}Tc-MAG3. Does that mean the mechanism of tubular handling is identical? Perhaps and perhaps not. The apical transporter also plays a role in moving the material from the cell. We can conclude with certainty now that ^{99m}Tc-MAG3 moves into the cell through a common transporter with PAH, although studies using intact animals had already given us an inkling of that information, but not so definitively. The effect of factors such as PAH, pH, protein binding, and diuresis on excretion of ^{99m}Tc-MAG3 and other compounds that investigate kidney function can still be judged by studying the intact animal (*17*).

So what does all this mean? In carrying out nuclear medicine procedures, we are studying molecular processes. The problem we face is defining the exact nature of the process we are observing. Current advances in molecular biology, with cloning techniques and the ability to express individual proteins that play a role in transporting specific or nonspecific substrate, give us great insight into how these compounds are handled and a more in-depth understanding of what it is that we are measuring. Have we obviated physiologic studies as well? At present, probably not. The intact organism is such a complexity of processes that no matter what we determine in vitro, surprises still lie ahead. Think about the many designer drugs that were based on preconceived notions of their function from in vitro models and that, when used in intact organisms, failed. However, we now have an effective means of studying and evaluating a wide variety of biologic processes. Molecular medicine and molecular imaging are here to stay. Let's not bury physiology quite yet.

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REFERENCES

1. Shikano N, Kanai Y, Kawai K, Ishikawa N, Endou H. Transport of ^{99m}Tc-MAG3 via rat renal organic anion transporter 1. *J Nucl Med.* 2004;45:80–85.
2. Smith H. *The Kidney: Structure and Function in Health and Disease.* New York, NY: Oxford University Press; 1951:1049.
3. Cross RJ, Taggart JV. Renal tubular transport: accumulation of p-aminohippurate by rabbit kidney slices. *Am J Physiol.* 1950;161:181–190.
4. Moller JV, Sheikh MI. Renal organic anion transport system: pharmacological, physiological, and biochemical aspects. *Pharmacol Rev.* 1983; 34:315–358.
5. Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. *Physiol Rev.* 1993;73:765–796.
6. Yee CA, Lee HB, Blaufox MD. Tc-99m DMSA renal uptake: influence of biochemical and physiologic factors. *J Nucl Med.* 1981;22:1054–1058.
7. Fritzberg AR, Kasina S, Eshima D, Johnson DL. Synthesis and biological evaluation of technetium-99m MAG3 as a hippuran replacement. *J Nucl Med.* 1986;27:111–116.
8. Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem.* 1997;272:18526–18529.
9. Sekine T, Cha SH, Endou H. The multispecific organic anion transporter (OAT) family. *Pflugers Arch July.* 2000;440:337–350.
10. Sweet DH, Wolff NA, Pritchard JB. Expression cloning and characterization of ROAT1. *J Biol Chem.* 1997;272:30088–30095.
11. Uchino H, Tamai I, Yamashita K, et al. p-Aminohippuric acid transport at renal apical membrane mediated by human inorganic phosphate transporter NPT1. *Biochem Biophys Res Commun.* 2000;270.:254–259.
12. Burckhardt G, Bahn A, Wolff NA. Molecular physiology of renal p-aminohippurate secretion. *News Physiol Sci.* 2001;16:114–118.
13. Lu R, Chan BS, Schuster VL. Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. *Am J Physiol.* 1999;276:F295–F303.
14. Race JE, Grassl SM, Williams WJ, Holtzman EJ. Molecular cloning and characterization of two novel human renal organic anion transporters (hOAT1 and hOAT3). *Biochem Biophys Res Commun.* 1999;255:508–514.
15. Roch-Ramel F. Renal transport of organic anions. *Curr Opin Nephrol Hypertens.* 1998;7:517–524.
16. Burckhardt G, Wolff NA, Bahn A. Molecular characterization of the renal organic anion transporter 1. *Cell Biochem Biophys.* 2002;36:169–174.
17. Lee HB, Blaufox MD. Mechanism of renal concentration of technetium-99m glucoheptonate. *J Nucl Med.* 1985;26:1308–1313.