

50th Anniversary Celebration Collection—Minireview

Drug Metabolism: A Half-Century Plus of Progress, Continued Needs, and New Opportunities

 F. Peter Guengerich

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee

Received October 27, 2021; accepted June 28, 2022

ABSTRACT

The systematic study of drug metabolism began in the 19th Century, but most of what we know now has been learned in the last 50 years. Drug metabolism continues to play a critical role in pharmaceutical development and clinical practice, as well as contributing to toxicology, chemical carcinogenesis, endocrinology, and drug abuse. The importance of the field will continue, but its nature will continue to develop with changes in analytical chemistry, structural biology, and artificial intelligence. Challenges and opportunities include toxicology, defining roles of genetic variations, and

application to clinical issues. Although the focus of this Minireview is cytochrome P450, the same principles apply to other enzymes and transporters involved in drug metabolism.

SIGNIFICANCE STATEMENT

Progress in the field of drug metabolism over the past 50 years has helped make the pharmaceutical enterprise what it is today. Drug metabolism will continue to be important. Challenges and opportunities for the future are discussed.

Introduction

I appreciate the editors' invitation to write a Minireview on the 50th anniversary of *Drug Metabolism and Disposition*. It is a fine journal that fills an important niche in this field. I continue to be a strong advocate of society-based journals managed by working scientists, published for the benefit of scientists, and have served as an associate editor for journals published by the American Society for Pharmacology and Experimental Therapeutics, the American Chemical Society, the American Association for Cancer Research, and the American Society for Biochemistry and Molecular Biology. I also add that this is (about) the 50th anniversary of the Gordon Research Conference on Drug Metabolism, another important entity in our discipline (with meeting delays caused by pandemic issues). A lot has happened in drug metabolism in the last 50 years, and I can only relate so much. The year 2022 also marks two other anniversaries for me. It is hard to believe that it has been 30 years since I received the American Society for Pharmacology and Experimental Therapeutics B. B. Brodie Award in Drug Metabolism. It has also been 20 years since I was awarded a Docteur Honoris Causa (honorary doctorate) from the University of Paris thanks to Professor Philippe Beaune and other friends in France.

As an undergraduate and graduate student, I had not started out to work on pharmaceutical problems or drugs. My interest began to develop when I did postdoctoral training with the late Professor Minor J. ("Jud") Coon on cytochrome P450 (P450) at the University of Michigan. I did not imagine that I would be working on the same enzymes for the rest of my career, but here I am. I was able to hold a good job

and provide for my family, and I also came to learn a lot about the pharmaceutical enterprise, which is absolutely vital to health in our society. Along the way I was able to make a few research contributions that have had some bearing on how drugs are developed. Just as importantly, I trained a number of young people who went on to advance the field in industry, academia, and administration; four of them have also contributed Minireview articles in this issue. Overall, I have been able to do a lot of interesting things and to meet many good people. For all of this, I am very grateful.

Changes and Progress

Fifty years ago, there was a heavy focus on studying drug metabolism in vivo in experimental animals. Predicting drug metabolism and pharmacokinetics was slow and hard, and often the animal work did not extrapolate well to humans. As I have said sometimes, people were good at making drugs to cure rats. Poor pharmacokinetics in humans was a major source of attrition for drug candidates, if not the major one (Prentis et al., 1988; Kola and Landis, 2004). Additionally, most drug doses were high, understandably, because in vitro pharmacology discovery programs were focused on getting IC_{50} and inhibition constant (K_i) values in the micromolar range, not nanomolar.

What did we know at the molecular level? When I joined Jud Coon's laboratory in 1973, no P450 had yet been purified to homogeneity. There was still debate about whether rats had one (hepatic) P450, or two, or more. For an interesting read, look at the published discussions following the talks at the first Microsomes and Drug Oxidations meeting in 1968. No one really knew much of anything about human P450s, except that they (or only a single one?) existed. There was no knowledge of even primary structures (sequences) of any P450s—recombinant DNA technology would not achieve this until 1982 (Fujii-Kuriyama et al., 1982). P450 induction had been discovered by Remmer (1959) and

This work was supported in part by the National Institute of General Medical Sciences (Grant R01-GM118122) (to F.P.G.).
dx.doi.org/10.1124/dmd.121.000739.

ABBREVIATIONS: AI, artificial intelligence; P450, cytochrome P450; SNV, single-nucleotide variation.

James and Elizabeth Miller, with their graduate student Allan Conney (Conney et al., 1956), but no one understood the mechanism nor was even sure if new protein synthesis was required.

Transporters were not even considered in the 1970s. As late as the early 1990s, the general dogma was that drugs are hydrophobic and enter cells by passive diffusion. Metabolites were usually more water-soluble than the parent drugs and therefore excreted, going back to some of the original concepts of R. T. Williams (Williams, 1947). Although active transport was known, it was largely studied in bacteria and unrecognized regarding a role in drug metabolism. Although a phenomenon known as multiple drug resistance was known to be operative in cancer cells and relevant to therapy (Endicott et al., 1987), the significance of these proteins in drug metabolism was not appreciated until later (Schuetz et al., 1995). Today, transporters are an important aspect of drug metabolism.

When I started my own laboratory at Vanderbilt in 1975, we focused on “classical” enzyme purification as a means to characterizing P450s. In retrospect, this was not terribly “hypothesis driven” work, but it was important at the time and fortunately, I did get funded. Our first efforts were directed toward rat liver P450s, and eventually we purified nine of them (Guengerich et al., 1982a; Larrey et al., 1984). We were not alone in this effort; we had some serious and stimulating competition (Ryan et al., 1975). With these P450s in hand, we could define catalytic specificity. In addition, we used antibodies (made in our own laboratory) and adapted newly discovered immunoelectrophoretic approaches (Towbin et al., 1979) (later termed “Western blotting”) to quantify individual P450s and reach some new conclusions about P450 regulation (Guengerich et al., 1982a,b; Dannan et al., 1983).

We were finding significant differences between the rat and rabbit P450s, and the quest to understand the human liver P450s became very pressing. At first, we were just purifying red proteins from columns and trying to see what these P450s would do, candidly speaking (Wang et al., 1980; Wang et al., 1983). We were finally able to obtain high quality human liver samples for our studies through some chance connections (Mukhopadhyay, 2012).

I was impressed with the research that Robert Smith was doing on pharmacogenetics at what was then St. Mary’s Hospital Medical School in London. His work on the polymorphism of debrisoquine 4-hydroxylation showed that a single P450 gene locus could be very dominant in the metabolism of a drug, i.e., there could be considerable specificity of an individual P450 for a substrate (Mahgoub et al., 1977; Tucker et al., 1977). Accordingly, we proceeded to use catalytic assays to monitor our purifications from liver. This approach was very technically challenging because of the presence of detergents and the difficulty of doing assays, such as debrisoquine hydroxylation (combined gas chromatography-mass spectrometry), but ultimately, we purified what are known today as P450s 1A2, 2A6, 2C8, 2C9, 2D6, and 3A4 from human liver (Distlerath et al., 1985; Guengerich et al., 1986; Shimada et al., 1986; Yun et al., 1991).

With these P450s in hand, plus antibodies we raised to them, we were able to define the selectivity of these P450s toward drugs, some steroids, and then many chemical carcinogens (Shimada et al., 1989). I suppose that all of this might have happened later anyway with recombinant DNA work and heterologous protein expression. By the same logic, one could dismiss Alexander Graham Bell’s invention of the telephone in that we no longer use it very much.

With this background and with much more work by many laboratories, particularly in the heterologous expression of P450s, it has been possible to learn much about 3-dimensional structures and mechanisms of induction and inhibition (Fig. 1). The Human Genome Project finally answered the question of how many P450 genes people have (57). I consider it very fortuitous that a small set of human liver P450s—1A2, 2C9, 2D6, 3A4 (vide supra), and 2C19—catalyze ~90% of the

drug oxidation reactions (Guengerich, 2015; Bhutani et al., 2021). Although this set does not work on all drugs, it could have been much more complicated if the situation were as complex as plant P450 biochemistry (>1,000 P450 genes in wheat, plus multiple reductases in plants).

Where Are We Today?

The knowledge about P450 has been very useful in several regards. Whereas I have focused on P450s, similar progress has been made with other enzymes involved in drug metabolism and with transporters. Logical comparisons can be made between human and experimental animals, which are still necessary in the contexts of understanding pharmacological actions and safety assessment. In vitro results with human enzyme systems can be used reasonably well to model and predict human pharmacokinetics and drug-drug interactions. Pharmacokinetic changes caused by single-nucleotide variants (SNVs) can be identified. The improvements in analytical chemistry over the past 50 years have been amazing, both in terms of the inherent capability, sensitivity, and throughput (especially NMR and particularly combined liquid chromatography-mass spectrometry, which is only ~35 years old). Our knowledge of chemical mechanisms of P450 reactions [particularly roles of Compound I (FeO^{3+})] has led to logical approaches to understanding metabolic pathways (Guengerich, 2001; Guengerich and Yoshimoto, 2018). We know a considerable amount about the auxiliary flavoprotein NADPH-P450 reductase, which delivers the electrons to most of the P450s, and also about adrenodoxin and cytochrome b_5 (which can deliver electrons in some cases), in terms of the basic chemistry and also the relevance of genetic variations in these proteins and their relevance to disease states. Of the 57 human P450s, at least 25 have one or more X-ray crystal structures [plus at least two good animal orthologs in cases where a human structure is not yet available (i.e., 4B1, 24A1)]. Overall, drug metabolism has been a real success story in the application of biochemical approaches to practical problems of pharmaceutical development, and I am thankful to have had at least some role in this over the past 47+ years.

A Continued Need for Drug Metabolism

The above description all sounds good, so do we still need drug metabolism and pharmacokinetics departments in the pharmaceutical industry and biochemists and pharmacologists studying basic research in drug metabolism? The answers are both, YES!

Oral, low molecular weight drugs will continue to be important. We have seen this recently with the coronavirus disease 2019 therapies. Today, many drugs are very potent (inhibition constant values in the low nanomolar range), practical, and are available at overall low cost (despite the naysayers, they actually have short patent lives on the market). Another reason for good drug metabolism science is that the regulatory expectations have become higher, e.g., US Food and Drug Administration, European Medicines Agency, Japanese Pharmaceuticals and Medical Devices Agency. With advances in analytical chemistry has come the expectation to define even more minor metabolites, and there are US Food and Drug Administration Metabolites in Safety Testing regulations regarding “disproportionate [human] metabolites” in species comparisons (Schadt et al., 2018). Time-dependent inhibition is still a problem, particularly with P450 3A4 (Eng et al., 2020). Related to this are drug-drug interactions, often with P450 3A4 and P-glycoprotein and some other transporters, which are still a problem, even fatal (Yu et al., 2018). Toxicology (safety assessment) problems are a major cause of attrition of drug candidates (Kola and Landis, 2004), particularly hepatic and cardiovascular issues. In particular, drug-induced liver injury is still difficult to predict and often involves drug metabolism.

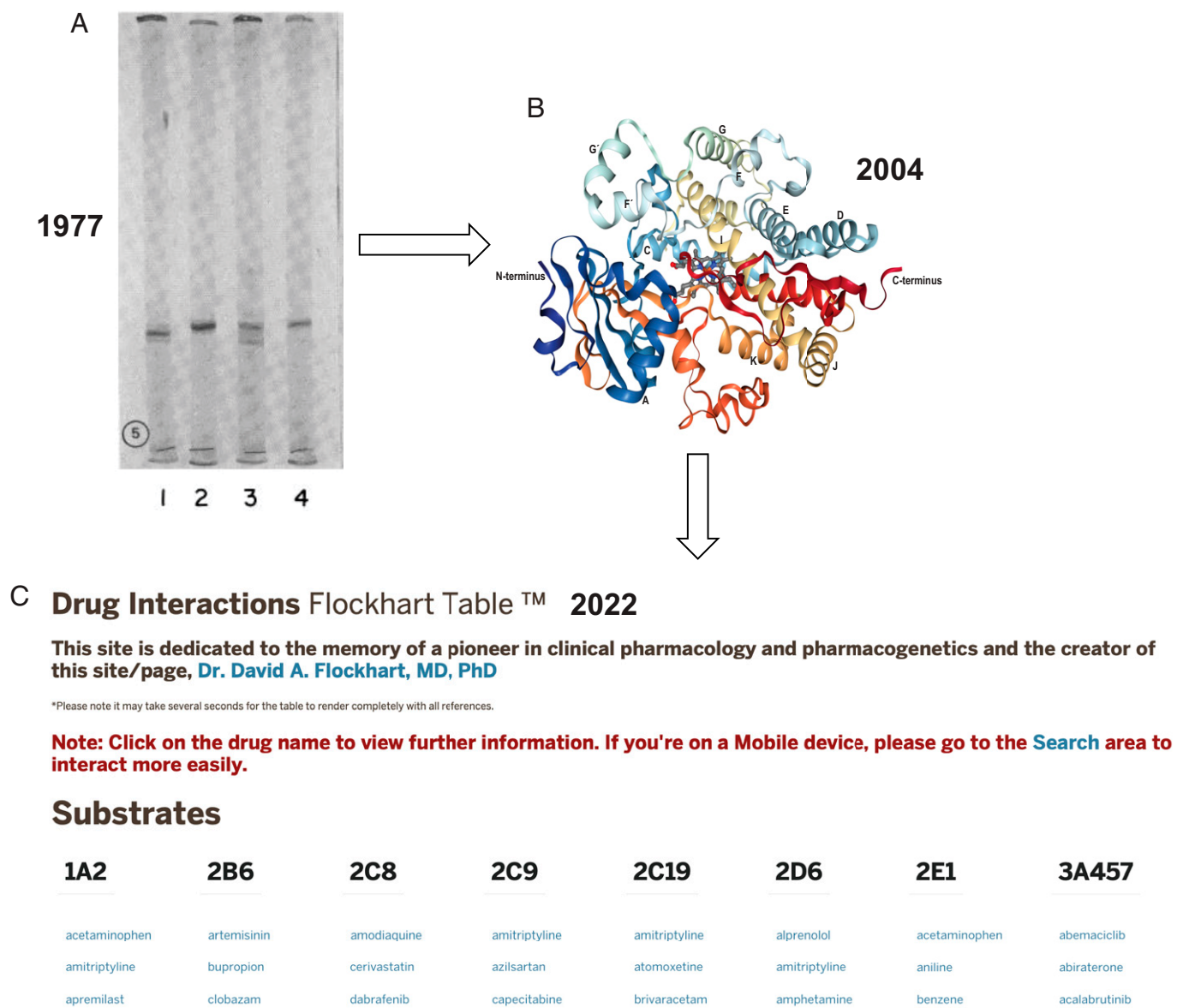


Fig. 1. Progress in drug metabolism. Dates are noted. (A) SDS—polyacrylamide gel electrophoresis (tube gels) of several rat P450s. Current names: lane 1, P450 2C11; lane 2, P450 2B1; lane 3, P450 2C6; lane 4, P450 2B2 (Guengerich, 1977). (B) Structure of P450 3A4 (no ligand). Protein Data Bank 1TQN (Yano et al., 2004). (C) Drug interaction table: <https://drug-interactions.medicine.iu.edu/MainTable.aspx> (accessed < June 11, 2022 >).

New Challenges and Opportunities

With every problem or challenge, there is an opportunity to make an important contribution. At the outset, I raise the caveat that the following are some of my ideas but that another person may well have a different list. Regardless, I seriously doubt that I will be able to solve all of these challenges myself in the time I have left to play the game. The future of drug metabolism is still bright for young scientists looking for meaningful careers.

Analytical chemistry will get even better. I am impressed with every new combined liquid chromatography-mass spectrometry model that comes out (but do not have the resources to buy). Even this may change though, at least the liquid chromatography component. UPLC can be fast but still takes time. Direct injection high resolution mass spectrometry approaches are already being used in metabolomics research (Sarvin et al., 2020). On the horizon is acoustic ejection mass spectrometry for very high throughput analysis, which has serious potential (Simon et al.,

2021; Zhang et al., 2021)—3600 samples/h with 0.01 μ l per shot! [In the P450 3A4 purification work (Guengerich et al., 1986) I thought I was doing well manually injecting one sample from a nifedipine oxidation reaction with a (P450) column fraction myself every 3 minutes, using the “new” 6.2 mm \times 80 mm Zorbax columns and running at a flow rate of 4 ml min^{-1} .] Another technique with potential is crystalline sponge X-ray diffraction analysis (Rosenberger et al., 2020; Rosenberger et al., 2021). A porous metal coordination complex functions as a host crystal, and μ g quantities of “guest” ligands can be added and induced to crystallize. The method has the power to determine 3-dimensional structures of drug metabolites with as little as 5 μ g, surpassing 2-D NMR in sensitivity and time for detailed analysis (Rosenberger et al., 2021), although only \sim 2/3 of the molecules tested are successful to date.

Another developing area is artificial intelligence (AI), which has already been employed extensively in predictions of genetic toxicology (Cunningham et al., 2004) and in regioselectivity of drug metabolism

(de Bruyn Kops et al., 2021). Most of the logic is based on literature precedents and AI, not on inherent structural docking and other physical principles. Although some of the algorithms have achieved impressive results (particularly in picking the top three “hot spots”), there are still many exceptions [e.g., hydroxylations of the angular methyl groups (C18, C19) of 4,5-dihydrotestosterone by P450 3A4 (Cheng et al., 2012)]. AI predictions of rates of metabolism are even more challenging, aside from Hammett series of molecules (Burka et al., 1985). Although, with enough dataset entries, even this might be possible someday.

Another area of potential is toxicity and safety assessment. Even predicting drug-drug interactions is difficult (Eng et al., 2020). These can be quite variable depending on the perpetrator and victim pair. Prediction of toxicities such as drug-induced liver injury is even more challenging because of the biologic complexity of tissues, even with *in vitro* data, but some progress is being made with AI approaches (Li et al., 2021). Predicting toxicities in other organs is also difficult, but some advances have been made in the context of biomarkers for both animals and humans (Harrill et al., 2009; Vazquez et al., 2020).

The field of pharmacogenetics developed with some of the enzymes in drug metabolism (Motulsky, 1957; Kalow, 1962) but accelerated with the work of Smith and others on what is now P450 2D6 (Mahgoub et al., 1977; Tucker et al., 1977; Caldwell, 2006). The input of molecular biology also changed the landscape (Nebert et al., 1981; Fujii-Kuriyama et al., 1982; Gonzalez et al., 1988). Although the early pharmacogenetic studies were interpreted in the context of “extensive” and “poor” metabolizers (fast and slow) (Mahgoub et al., 1977), today we know that there are hundreds of SNVs with at least some of the P450s (<https://www.pharmvar.org/gene/CYP2D6>), and bimodal interpretation of the results is much too simplistic. We also know that >100 clinically relevant SNVs of the steroid 21-hydroxylase P450 21A2 exist (grouped into three phenotypic categories) (Wang et al., 2017). Understanding the links between single amino acid structural changes and function in these (and other) enzymes is challenging, and few natural P450 variants have been crystallized (Parikh et al., 2020). The root of the problem is seen in the application of the Eyring equation

$$k_{\text{obs}} = (RT/N_A h) e^{-\Delta G^\ddagger/RT}$$

(where R is the universal gas constant, T is the absolute temperature, N_A is Avagadro’s number, and h is Planck’s constant), in that a 10-fold variation in enzyme activity (k_{obs}) is linked with a free energy change (ΔG) of 1.3 kcal mol⁻¹, less than a single hydrogen bond (Wang et al., 2017). Can we ever understand or predict rates of drug metabolism of variants? Also, realize that different coding region SNVs can yield different changes with different drug oxidation reactions (Takanashi et al., 2000). At a clinical level, there is still limited use of what we do know about phenotypes of SNVs, with the most prominent examples being thiopurine *S*-methylation (Lennard, 2014), warfarin/P450 2C9 (Higashi et al., 2002), and clopidogrel/P450 2C19 (Pare et al., 2010). Some use of P450 2D6 SNVs has been made with iloperidone (FANAPT) (<https://fanaptpro.com/wp-content/uploads/2015/02/Fanapt-Prescribing-Information.pdf>) and possibly other neurological drugs (Haslemo et al., 2019). The SNVs in steroid-metabolizing P450s have more dramatic clinical consequences (Miller and Auchus, 2011), but the effects of new SNVs are still rather unpredictable. For instance, development of drugs that can rescue poor phenotypes of the steroid 21-hydroxylase P450 21A2 is a formidable challenge.

Although some functions are now associated with most of the human P450s, there are a few recalcitrant “orphans” (Guengerich, 2015), e.g., 4X1 and 20A1. Beyond this, there is still a concern about what the relatively slow rate of fatty acid oxidations catalyzed by some of the P450s really mean (Stark et al., 2008; Fekry et al., 2019). Are we missing

important roles? Are the functions of the xenobiotic-metabolizing P450s only general cellular protection (e.g., against ingested natural products) or do more of these P450s have physiologically relevant substrates?

Of the 57 human P450s, 50 are inherently microsomal and seven are mitochondrial (Guengerich, 2015). Most of the mitochondrial P450s seem to all be essential (27C1?) and have important physiologic substrates, but we also know that at least some of these can have roles in drug metabolism and even bioactivation (Zhang et al., 2012; Rendic and Guengerich, 2018). Are there more drugs oxidized in mitochondria? Narayan Avadhani and his associates have shown that some of the microsomal P450s can also be modified and relocate to the mitochondria (Avadhani et al., 2011). They appear to then use adrenodoxin as a source of electrons for reduction, but we know little about the details of these interactions. There are still major questions about the abundance of the mitochondrial P450s and their accessory proteins in the relevant zones of the extrahepatic tissues where they are found.

Although we are developing concepts of how P450s interact with NADPH-P450 reductase (Cheng et al., 2021) and the relevance of genetic variations in these proteins and their relevance to disease states is recognized (Riddick et al., 2013; Burkhard et al., 2017), there are other interactions that remain poorly understood. The interaction of adrenodoxin has only been studied with some of the mitochondrial P450s (Lambeth and Kriengsiri, 1985; Beilke et al., 2002; Brixius-Anderko and Scott, 2021; Glass et al., 2021). Although it has been >50 years since the discovery of a role for cytochrome *b*₅ in a P450 reaction (Hildebrandt and Estabrook, 1971; Guengerich, 2022), there are still major questions about mechanisms and relevance *in vivo* (McLaughlin et al., 2010). *b*₅ may stimulate or inhibit P450 reactions and is most essential in the P450 17A1 lyase reaction (Katagiri et al., 1995). Stimulation may or may not involve electron transfer with individual P450s (Yamazaki et al., 2002). Recently retinoid-binding proteins have been shown to deliver retinoids directly to P450 Family 26 enzymes (Zhong et al., 2018) and P450 27C1 (Glass and Guengerich, 2021), although structural details are unknown. The demonstrated roles of binding proteins of the retinoid-metabolizing P450s raise the question of whether fatty acid binding proteins may also be involved in substrate delivery. There is still a glaring need for more structural work on binary complexes of P450s with accessory proteins.

The clinical relevance of P450s has been clearly demonstrated in drug-drug interactions and endocrinology, but the situation is less clear in other areas. Chemical carcinogenesis was one of the areas that fueled much of the early research with P450s and several other drug-metabolizing enzymes. Although roles for many of these enzymes have been clearly implicated in animal models (Guengerich, 1988), the situation is less clear in humans and studies of SNVs have not been very definitive. Unfortunately, interest in this area has waned, certainly with the funders at the National Institutes of Health and much of the cancer research community. Other areas that have shown some potential relevance of P450s and SNVs include hypertension (Gainer et al., 2005) and neurologic disease (Cheng et al., 2013).

P450s can also be drug targets, e.g., P450s 5A1, 11B2, 17A1, 19A1. Inhibition of P450 19A1 steroid aromatase activity is an established means of treating breast and other estrogen-dependent cancers. Some other P450s are drug targets and have drugs to inhibit them, but these could be improved, e.g., P450 17A1 (Bird and Abbott, 2016). More P450s, including those essential in normal settings, are potential drug targets. Finally, inhibiting P450s of parasites and other infectious agents is an established approach to treating very important disease problems, e.g., fungal infections, and frequent targets are P450 51 enzymes (Frigeri et al., 2014). P450s have also been considered as targets in treating tuberculosis (McLean et al., 2007).

Other Issues Related To Drug Metabolism

There are several “nonscience” issues of concern in the area of drug metabolism, although they really do involve science.

First, the past two years have shown us how fragile our interactive networks can be. There are new approaches to communication, but the live meetings have been sorely missed. Some of the important things I have to look back on in my career are the lessons I learned in my travels and the people I have met. We had to postpone the biennial International Conference on Cytochrome P450 (ICCP450) from 2021 to 2022, which has now occurred by the time this review is published. I worry about both diseases and international politics disrupting future meetings, not only ICCP450 but also others.

Scientific publishing has changed considerably in recent years. The challenges have been great for historic society-based journals (e.g., *Drug Metabolism and Disposition*, *The Journal of Biologic Chemistry*). Although the development of electronic capability has been very useful, economic pressures have been an issue. Will journals edited by working scientists survive? Will books exist or have they become dinosaurs (the offices of our junior faculty certainly do not resemble mine!)? I have serious concerns about the development of nonreviewed publications (which will remain nameless here). Exactly what is scientific communication going to look like in 50 more years?

Finally, who will be training the next generation of drug metabolism scientists? I see contemporaries disappearing but limited enthusiasm of universities for hiring in the area. Would I even be hired today? What do we need to be teaching graduate students and postdocs to prepare them for the pharmaceutical industry and other careers? What new subject areas need to be added to the repertoire of a trainee—and which basic ones need to be retained? Who will be the faculty doing this and, most importantly, will the National Institutes of Health and other agencies fund individuals to maintain a viable cadre in this field?

Conclusions

The science of drug metabolism really began in the 19th Century (Caldwell, 2006; Guengerich, 2018) (see also <https://www.issx.org/page/History>), but the bulk of what we know has been learned in the last 50 years. I have been privileged to have had a role in this. Drug metabolism has been a classic success in the application of basic science to important health problems. For reasons described, drug metabolism will continue to play a vital role in the process of discovering and developing drugs. A number of opportunities exist for further development. There are scientific and other challenges ahead, as there were in the past.

Acknowledgments

I thank K. Trisler for her assistance in the preparation of the manuscript and the editors for the opportunity to contribute this Minireview. Finally, I thank the 22 graduate students, 141 postdocs and visiting scientists, and all others who have worked in this laboratory and contributed, as well as our collaborators. Their individual efforts have made our research possible but are too many to mention in the available space.

Finally, I would like to dedicate this article to the memory of Prof. Michael R. Waterman, my friend and colleague for over 20 years. Mike died in November 2021 during the start of this draft. He made many contributions to the field of P450 research and is missed by all who knew him.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Guengerich.

References

- Gillette JR, Conney AH, Cosmides GJ, Estabrook RW, Fouts JR, and Mannering GJ, eds (1968) in *Microsomes and Drug Oxidations*, pp 3–547. Academic Press, Bethesda, MD.
- Avadhani NG, Sangar MC, Bansal S, and Bajpai P (2011) Bimodal targeting of cytochrome P450s to endoplasmic reticulum and mitochondria: the concept of chimeric signals. *FEBS J* **278**:4218–4229.
- Beilke D, Weiss R, Löhner F, Pristovsek P, Hannemann F, Bernhardt R, and Rüterjans H (2002) A new electron transport mechanism in mitochondrial steroid hydroxylase systems based on structural changes upon the reduction of adrenodoxin. *Biochemistry* **41**:7969–7978.
- Bhutani P, Joshi G, Raja N, Bachhav N, Rajanna PK, Bhutani H, Paul AT, and Kumar R (2021) US FDA approved drugs from 2015–June 2020: a perspective. *J Med Chem* **64**:2339–2381.
- Bird IM and Abbott DH (2016) The hunt for a selective 17,20 lyase inhibitor; learning lessons from nature. *J Steroid Biochem Mol Biol* **163**:136–146.
- Brixius-Anderko S and Scott EE (2021) Structural and functional insights into aldosterone synthase interaction with its redox partner protein adrenodoxin. *J Biol Chem* **296**:100794.
- Burka LT, Guengerich FP, Willard RJ, and Macdonald TL (1985) Mechanism of cytochrome P-450 catalysis. mechanism of *N*-dealkylation and amine oxide deoxygenation. *J Am Chem Soc* **107**:2549–2551.
- Burkhard FZ, Parween S, Udhane SS, Flück CE, and Pandey AV (2017) P450 Oxidoreductase deficiency: analysis of mutations and polymorphisms. *J Steroid Biochem Mol Biol* **165**:38–50.
- Caldwell J (2006) Drug metabolism and pharmacogenetics: the British contribution to fields of international significance. *Br J Pharmacol* **147**:S89–S99.
- Cheng J, Zhen Y, Miksys S, Beyoğlu D, Krausz KW, Tyndale RF, Yu A, Idle JR, and Gonzalez FJ (2013) Potential role of CYP2D6 in the central nervous system. *Xenobiotica* **43**:973–984.
- Cheng Q, Sohl CD, Yoshimoto FK, and Guengerich FP (2012) Oxidation of dihydrotestosterone by human cytochromes P450 19A1 and 3A4. *J Biol Chem* **287**:29554–29567.
- Cheng S, Bo Z, Hollenberg P, Osawa Y, and Zhang H (2021) Amphipol-facilitated elucidation of the functional tetrameric complex of full-length cytochrome P450 CYP2B4 and NADPH-cytochrome P450 oxidoreductase. *J Biol Chem* **296**:100645.
- Conney AH, Miller EC, and Miller JA (1956) The metabolism of methylated aminoazo dyes. V. evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Res* **16**:450–459.
- Cunningham AR, Cunningham SL, and Rosenkranz HS (2004) Structure-activity approach to the identification of environmental estrogens: the MCASE approach. *SAR QSAR Environ Res* **15**:55–67.
- Dannan GA, Guengerich FP, Kaminsky LS, and Aust SD (1983) Regulation of cytochrome P-450. Immunohistochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl congeners. *J Biol Chem* **258**:1282–1288.
- de Bruyn Kops C, Sicho M, Mazzolari A, and Kirchmair J (2021) GLORYx: prediction of the metabolites resulting from phase 1 and phase 2 biotransformations of xenobiotics. *Chem Res Toxicol* **34**:286–299.
- Distlerath LM, Reilly PE, Martin MV, Davis GG, Wilkinson GR, and Guengerich FP (1985) Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin *O*-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* **260**:9057–9067.
- Endicott JA, Juranka PF, Sarangi F, Gerlach JH, Deuchars KL, and Ling V (1987) Simultaneous expression of two P-glycoprotein genes in drug-sensitive Chinese hamster ovary cells. *Mol Cell Biol* **7**:4075–4081.
- Eng H, Tseng E, Cerny MA, Goosen TC, and Obach RS (2020) Cytochrome P450 3A time-dependent inhibition assays are too sensitive for identification of drugs causing clinically significant drug-drug interactions: a comparison of human liver microsomes and hepatocytes and definition of boundaries for inactivation rate constants. *Drug Metab Dispos* **49**:442–450.
- Fekry MI, Xiao Y, Berg JZ, and Guengerich FP (2019) A role for the orphan human cytochrome P450 2S1 in polyunsaturated fatty acid ω -1 hydroxylation using an untargeted metabolomic approach. *Drug Metab Dispos* **47**:1325–1332.
- Friggeri L, Hargrove TY, Rachakonda G, Williams AD, Wawrzak Z, Di Santo R, De Vita D, Waterman MR, Tortorella S, Villalta F et al. (2014) Structural basis for rational design of inhibitors targeting *Trypanosoma cruzi* sterol 14 α -demethylase: two regions of the enzyme molecule potentiate its inhibition. *J Med Chem* **57**:6704–6717.
- Fujii-Kuriyama Y, Mizukami Y, Kawajiri K, Sogawa K, and Muramatsu M (1982) Primary structure of a cytochrome P-450: coding nucleotide sequence of phenobarbital-inducible cytochrome P-450 cDNA from rat liver. *Proc Natl Acad Sci USA* **79**:2793–2797.
- Gainer JV, Bellamine A, Dawson EP, Womble KE, Grant SW, Wang Y, Cupples LA, Guo CY, Demissie S, O'Donnell CJ et al. (2005) Functional variant of CYP4A11 20-hydroxycoisatetraenoic acid synthase is associated with essential hypertension. *Circulation* **111**:63–69.
- Glass SM and Guengerich FP (2021) Cellular retinoid-binding proteins transfer retinoids to human cytochrome P450 27C1 for desaturation. *J Biol Chem* **297**:101142.
- Glass SM, Webb SN, and Guengerich FP (2021) Binding of cytochrome P450 27C1, a retinoid desaturase, to its accessory protein adrenodoxin. *Arch Biochem Biophys* **714**:109076.
- Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, and Meyer UA (1988) Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* **331**:442–446.
- Guengerich FP (1977) Separation and purification of multiple forms of microsomal cytochrome P-450. Activities of different forms of cytochrome P-450 towards several compounds of environmental interest. *J Biol Chem* **252**:3970–3979.
- Guengerich FP (1988) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* **48**:2946–2954.
- Guengerich FP (2001) Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* **14**:611–650.
- Guengerich FP (2015) Human cytochrome P450 enzymes, in: *Cytochrome P450: Structure, Mechanism, and Biochemistry* (Ortiz de Montellano PR, ed), pp 523–785. Springer, New York.
- Guengerich FP (2018) Introduction and historical perspective, in *Biotransformation* (Guengerich FP, ed) pp 1–7, Elsevier, Oxford, UK.
- Guengerich FP (2022) On ‘Evidence for the participation of cytochrome b₅ in hepatic microsomal mixed-function oxidation reactions’ by Alfred Hildebrandt and Ronald W. Estabrook *Arch Biochem Biophys* **726**:109177.

- Guengerich FP, Dannan GA, Wright ST, Martin MV, and Kaminsky LS (1982a) Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β -naphthoflavone. *Biochemistry* **21**:6019–6030.
- Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, and Waxman DJ (1986) Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* **261**:5051–5060.
- Guengerich FP, Wang P, and Davidson NK (1982b) Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry* **21**:1698–1706.
- Guengerich FP and Yoshimoto FK (2018) Formation and cleavage of C-C bonds by enzymatic oxidation-reduction reactions. *Chem Rev* **118**:6573–6655.
- Harrill AH, Ross PK, Gatti DM, Threadgill DW, and Rusyn I (2009) Population-based discovery of toxicogenomics biomarkers for hepatotoxicity using a laboratory strain diversity panel. *Toxicol Sci* **110**:235–243.
- Haslemo T, Eliasson E, Jukić MM, Ingelman-Sundberg M, and Molden E (2019) Significantly lower CYP2D6 metabolism measured as the O/N-desmethylvenlafaxine metabolic ratio in carriers of CYP2D6*41 versus CYP2D6*9 or CYP2D6*10: a study on therapeutic drug monitoring data from 1003 genotyped Scandinavian patients. *Br J Clin Pharmacol* **85**:194–201.
- Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, and Rettie AE (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* **287**:1690–1698.
- Hildebrandt A and Estabrook RW (1971) Evidence for the participation of cytochrome b_5 in hepatic microsomal mixed-function oxidation reactions. *Arch Biochem Biophys* **143**:66–79.
- Kalow W (1962) *Pharmacogenetics*, W. B. Saunders, Philadelphia.
- Katagiri M, Kagawa N, and Waterman MR (1995) The role of cytochrome b_5 in the biosynthesis of androgens by human P450c17. *Arch Biochem Biophys* **317**:343–347.
- Kola I and Landis J (2004) Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* **3**:711–715.
- Lambeth JD and Kriengsiri S (1985) Cytochrome P-450_{sc}-adrenodoxin interactions. Ionic effects on binding, and regulation of cytochrome reduction by bound steroid substrates. *J Biol Chem* **260**:8810–8816.
- Larrey D, Distlerath LM, Dannan GA, Wilkinson GR, and Guengerich FP (1984) Purification and characterization of the rat liver microsomal cytochrome P-450 involved in the 4-hydroxylation of debrisoquine, a prototype for genetic variation in oxidative drug metabolism. *Biochemistry* **23**:2787–2795.
- Lennard L (2014) Implementation of TPMT testing. *Br J Clin Pharmacol* **77**:704–714.
- Li T, Tong W, Roberts R, Liu Z, and Thakkar S (2021) DeepDILL: deep learning-powered drug-induced liver injury prediction using model-level representation. *Chem Res Toxicol* **34**:550–565.
- Mahgoub A, Idle JR, Dring LG, Lancaster R, and Smith RL (1977) Polymorphic hydroxylation of debrisoquine in man. *Lancet* **2**:584–586.
- McLaughlin LA, Ronseaux S, Finn RD, Henderson CJ, and Wolf CR (2010) Deletion of microsomal cytochrome b_5 profoundly affects hepatic and extrahepatic drug metabolism. *Mol Pharmacol* **78**:269–278.
- McLean KJ, Dunford AJ, Neeli R, Driscoll MD, and Munro AW (2007) Structure, function and drug targeting in *Mycobacterium tuberculosis* cytochrome P450 systems. *Arch Biochem Biophys* **464**:228–240.
- Miller WL and Auchus RJ (2011) The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev* **32**:81–151.
- Motulsky AG (1957) Drug reactions enzymes, and biochemical genetics. *J Am Med Assoc* **165**:835–837.
- Mukhopadhyay R (2012) Human cytochrome P450s: the work of Frederick Peter Guengerich. *J Biol Chem* **287**:15798–15800.
- Nebert DW, Eisen HJ, Negishi M, Lang MA, Hjelmeland LM, and Okey AB (1981) Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. *Annu Rev Pharmacol Toxicol* **21**:431–462.
- Paré G, Mehta SR, Yusuf S, Anand SS, Connolly SJ, Hirsh J, Simonsen K, Bhatt DL, Fox KA, and Eikelboom JW (2010) Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. *N Engl J Med* **363**:1704–1714.
- Pariikh SJ, Evans CM, Obi JO, Zhang Q, Maekawa K, Glass KC, and Shah MB (2020) Structure of cytochrome P450 2C9*2 in complex with an anti-hypertensive drug losartan: Insights into the effect of genetic polymorphism. *Mol Pharmacol* **98**:529–539.
- Prentiss RA, Lis Y, and Walker SR (1988) Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964–1985). *Br J Clin Pharmacol* **25**:387–396.
- Remmer H (1959) [The acceleration of evipan oxidation and the demethylation of methylaminopyrine by barbiturates]. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* **237**:296–307.
- Rendić SP and Peter Guengerich F (2018) Human cytochrome P450 enzymes 5-51 as targets of drugs and natural and environmental compounds: mechanisms, induction, and inhibition - toxic effects and benefits. *Drug Metab Rev* **50**:256–342.
- Riddick DS, Ding X, Wolf CR, Porter TD, Pandey AV, Zhang Q-Y, Gu J, Finn RD, Ronseaux S, McLaughlin LA et al. (2013) NADPH-cytochrome P450 oxidoreductase: roles in physiology, pharmacology, and toxicology. *Drug Metab Dispos* **41**:12–23.
- Rosenberger L, von Essen C, Khutia A, Kühn C, Georgi K, Hirsch AKH, Hartmann RW, and Badolo L (2021) Crystalline sponge affinity screening: a fast tool for soaking condition optimization without the need of X-ray diffraction analysis. *Eur J Pharm Sci* **164**:105884.
- Rosenberger L, von Essen C, Khutia A, Kühn C, Urbahns K, Georgi K, Hartmann RW, and Badolo L (2020) Crystalline sponges as a sensitive and fast method for metabolite identification: application to gemfibrozil and its phase I and II metabolites. *Drug Metab Dispos* **48**:587–593.
- Ryan D, Lu AYH, West S, and Levin W (1975) Multiple forms of cytochrome P-450 in phenobarbital- and 3-methylcholanthrene-treated rats. Separation and spectral properties. *J Biol Chem* **250**:2157–2163.
- Sarvin B, Lagziel S, Sarvin N, Mukha D, Kumar P, Aizenshtein E, and Shlomi T (2020) Fast and sensitive flow-injection mass spectrometry metabolomics by analyzing sample-specific ion distributions. *Nat Commun* **11**:3186.
- Schadt S, Bister B, Chowdhury SK, Funk C, Hop CECA, Humphreys WG, Igarashi F, James AD, Kagan M, Khojasteh SC, et al. (2018) A decade in the MIST: learnings from investigations of drug metabolites in drug development under the “Metabolites in Safety Testing” regulatory guidance. *Drug Metab Dispos* **46**:865–878.
- Schuetz EG, Furuya KN, and Schuetz JD (1995) Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther* **275**:1011–1018.
- Shimada T, Iwasaki M, Martin MV, and Guengerich FP (1989) Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA 1535/pSK1002. *Cancer Res* **49**:3218–3228.
- Shimada T, Misono KS, and Guengerich FP (1986) Human liver microsomal cytochrome P-450 mephenytoin 4-hydroxylase, a prototype of genetic polymorphism in oxidative drug metabolism. Purification and characterization of two similar forms involved in the reaction. *J Biol Chem* **261**:909–921.
- Simon RP, Häbe TT, Ries R, Winter M, Wang Y, Fernández-Montalván A, Bischoff D, Runge F, Reindl W, Luippold AH, et al. (2021) Acoustic ejection mass spectrometry: a fully automatable technology for high-throughput screening in drug discovery. *SLAS Discov* **26**:961–973.
- Stark K, Dostalek M, and Guengerich FP (2008) Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide. *FEBS J* **275**:3706–3717.
- Takanashi K, Tainaka H, Kobayashi K, Yasumori T, Hosakawa M, and Chiha K (2000) CYP2C9 Ile³⁵⁹ and Leu³⁵⁹ variants: enzyme kinetic study with seven substrates. *Pharmacogenetics* **10**:95–104.
- Towbin H, Staehelin T, and Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76**:4350–4354.
- Tucker GT, Silas JH, Iyuu AO, Lennard MS, and Smith AJ (1977) Polymorphic hydroxylation of debrisoquine. *Lancet* **2**:718.
- Vazquez JH, Clemens MM, Allard FD, Yee EU, Kennon-McGill S, Mackintosh SG, Jaeschke H, Hambuchen MD, and McGill MR (2020) Identification of serum biomarkers to distinguish hazardous and benign aminotransferase elevations. *Toxicol Sci* **173**:244–254.
- Wang C, Pallan PS, Zhang W, Lei L, Yoshimoto FK, Waterman MR, Egli M, and Guengerich FP (2017) Functional analysis of human cytochrome P450 21A2 variants involved in congenital adrenal hyperplasia. *J Biol Chem* **292**:10767–10778.
- Wang P, Mason PS, and Guengerich FP (1980) Purification of human liver cytochrome P-450 and comparison to the enzyme isolated from rat liver. *Arch Biochem Biophys* **199**:206–219.
- Wang PP, Beaune P, Kaminsky LS, Dannan GA, Kadlubar FF, Larrey D, and Guengerich FP (1983) Purification and characterization of six cytochrome P-450 isozymes from human liver microsomes. *Biochemistry* **22**:5375–5383.
- Williams RT (1947) *Detoxication Mechanisms*, Wiley, New York.
- Yamazaki H, Nakamura M, Komatsu T, Ohyama K, Hatanaka N, Asahi S, Shimada N, Guengerich FP, Shimada T, Nakajima M et al. (2002) Roles of NADPH-P450 reductase and apo- and holo-cytochrome b_5 on xenobiotic oxidations catalyzed by 12 recombinant human cytochrome P450s expressed in membranes of *Escherichia coli*. *Protein Expr Purif* **24**:329–337.
- Yano JK, Wester MR, Schoch GA, Griffin KJ, Stout CD, and Johnson EF (2004) The structure of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-Å resolution. *J Biol Chem* **279**:38091–38094.
- Yu J, Zhou Z, Tay-Sontheimer J, Levy RH, and Ragueneau-Majlessi I (2018) Risk of clinically relevant pharmacokinetic-based drug-drug interactions with drugs approved by the US Food and Drug Administration between 2013 and 2016. *Drug Metab Dispos* **46**:835–845.
- Yun CH, Shimada T, and Guengerich FP (1991) Purification and characterization of human liver microsomal cytochrome P-450 2A6. *Mol Pharmacol* **40**:679–685.
- Zhang D, Flint O, Wang L, Gupta A, Westhouse RA, Zhao W, Raghavan N, Caceres-Cortes J, Marathe P, Shen G, et al. (2012) Cytochrome P450 11A1 bioactivation of a kinase inhibitor in rats: use of radioprofiling, modulation of metabolism, and adrenocortical cell lines to evaluate adrenal toxicity. *Chem Res Toxicol* **25**:556–571.
- Zhang H, Liu C, Hua W, Ghislain LP, Liu J, Aschenbrenner L, Noell S, Dirico KJ, Lanyon LF, Steppan CM, et al. (2021) Acoustic ejection mass spectrometry for high-throughput analysis. *Anal Chem* **93**:10850–10861.
- Zhong G, Ortiz D, Zelter A, Nath A, and Isoherranen N (2018) CYP26C1 is a hydroxylase of multiple active retinoids and interacts with cellular retinoic acid binding proteins. *Mol Pharmacol* **93**:489–503.

Address correspondence to: F. Peter Guengerich, Department of Biochemistry, Vanderbilt University School of Medicine, 2200 Pierce Avenue, 638 Robinson Research Building, Nashville, Tennessee 37232-0146. E-mail: f.guengerich@vanderbilt.edu