This article will examine these phases and the evolving direction of drug metabolism in drug discovery and development. We will enter a new era, which I will call the “biology” phase. The science of drug metabolism, like any other science, has advanced from simple beginnings (by today’s standards) to its present state. One can examine the path that has been taken to understand the forces driving the direction of evolution of this science. The trends discovered can then be used to make reasonable extrapolations about the changes that might be expected in the future. That exercise is the subject of this article. The main focus will be on drug metabolism as practiced in the industrial environment, representing the author’s main experience as well as the principal arena of practical applications of the science. The discussion will draw mainly on broad phenomena occurring in this application of drug metabolism to drug discovery and development.

If we take a long view of the history of the use of drug metabolism (DM) studies in industrial drug discovery and development, we can see several phases. For many years, companies developing new drugs did not pay any attention to the question of the metabolic fate of the compound. Indeed, the question was not even asked. Later, as regulatory agencies began to expect a more complete accounting of the actions and disposition of new drugs submitted for marketing approval, companies introduced the isolation and identification of metabolites recovered from urine and feces. We may call this the “chemistry” phase. This phase was largely descriptive, with little understanding of the phenomena leading to certain metabolic pathways except the purely chemical properties of the drug compound itself. Then, in the 1970s, we entered the “biochemistry” phase, which allows us to account for and even predict interspecies differences, individual variation, and drug-drug interactions occurring because of drug-metabolizing enzymes. Presently, we are watching the development of the “genetics” phase, in which various technologies are being introduced to allow industry to take into consideration the genetic determinants of drug metabolism during the preclinical selection and clinical evaluation of new drug candidates. Interestingly, this is not a process of serial replacement of one approach by the next, because nothing has become obsolete: all phases continue to coexist. For instance, the isolation and identification of metabolites is even more important today than it was in the past.

What, then, is the next phase? For this, one must look into the ultimate destination of medicine. In other words, what will therapeutics look like in the future? I believe that in the first quarter of the 21st century, we will enter a new era, which I will call the “biology” phase. This article will examine these phases and the evolving direction of DM, especially as it is applied to industrial drug discovery and development.

Discussion

Present Practices. A good place to start is to consider the role of DM in present-day drug development. We can consider that DM is involved in three distinct areas of the process: discovery, nonclinical development, and clinical development. Although DM and pharmacokinetics (PK) are interrelated, they are nonetheless distinct, and this discussion will not consider PK except as the consequence of DM.

First, consider the discovery phase. After identification of a novel therapeutic target (e.g., an enzyme to be inhibited or a receptor to be antagonized), a discovery program needs to demonstrate proof-of-principle in a predictive animal pharmacological model. It is common for this stage to be delayed for reasons of poor efficacy of the lead compound in the animal model, despite good in vitro potency. Among the several reasons that the desired pharmacology might not be expressed in the animal model is rapid metabolism, resulting in low exposure of the target tissue to the test compound. Recognition that metabolism limits exposure (by experiments showing low plasma and tissue levels, as well as excretion of substantial portions of the dose as metabolite) leads next to identification of the pathway of metabolism and the structural site of chemical modification of the test compound (by modern methods of chromatography and spectroscopic structure elucidation, most notably liquid chromatography/mass spectrometry and liquid chromatography/nuclear magnetic resonance). Having identified the metabolic “hot spot,” the DM scientist collaborates with the synthetic-medicine chemist to design several analogs to be more metabolically stable than the original lead compound. A combination of in vitro (e.g., liver microsomes) and in vivo methods are used to determine which, if any, of the analogs has been successfully stabilized. Dosing with the metabolically improved analog normally results in expression of the desired pharmacology, allowing the program to move into the phase of optimization of activity. Typically, hundreds or even thousands of analogs are synthesized and tested in vitro against the pharmacological target protein as...
medicinal chemistry establishes the structure-activity relationship for this chemotype and learns how to make extremely potent analogs. During this process, in which new metabolically labile groups might be introduced into the chemotype, DM has the important function of determining metabolic liability of the new potent analogs to ensure that the molecules resulting from activity optimization retain favorable metabolic characteristics. The advent of combinatorial chemistry, with its potential to generate large numbers of pharmaceutically potent compounds, has produced a severe challenge to the DM scientist to devise reliable methods of assessing metabolic stability with throughput rates adequate to provide nearly real-time feedback to chemistry. The solutions, although varied, generally fall into two types: either higher throughput in vitro metabolic systems such as liver microsomes, or accelerated in vivo systems such as simultaneous multicom pound dosing to rats or dogs, with liquid chromatography/mass spectrometry/mass spectrometry analysis allowing deconvolution of the data (Berman et al., 1997; Olah et al., 1997).

The result of this optimization phase is a clinical candidate that must be assessed for its “developability” with respect to DM (i.e., determining whether favorable DM and PK characteristics in the animal models will also be displayed in humans). This assessment involves using animal in vitro and in vivo data combined with human in vitro data to determine qualitatively whether humans will eliminate the clinical candidate via the same pathways as did the animal models and to quantitatively (or at least semiquantitatively) estimate the overall clearance rate attributable to those pathways (Carlile et al., 1997; Iwatsubo et al., 1997). Many companies also establish the human P450 enzyme profile at this point to anticipate the following:

(a) potential drug-drug interactions involving competition for shared P450 enzymes (e.g., CYP 3A4);
(b) individual variability due to highly variable or polymorphic P450 enzymes (e.g., CYP 2C9 or 2D6); and
(c) probable P450 enzyme induction (e.g., CYP 1A2).

Once the study moves into the clinic, Phase I normally includes an ADME study with the 14C-labeled drug, which allows a comparison of the actual human in vivo metabolic pathways with those determined in the animal toxicology species. Discovery of substantial amounts of a circulating metabolite in humans that was not present in any of the toxicological species means that the human subjects are being exposed to a compound with unknown toxic potential. Often this will result in a hiatus of the clinical work until an appropriate animal species can be tested with the synthesized metabolite. Thus the metabolite-structure elucidation process of the clinical ADME study needs to proceed quickly and metabolite standards synthesized promptly for toxicology dosing, if necessary, and for metabolite-assay development. To minimize the chance of having to put the clinical study on hold, most companies strive to select a preclinical toxicological animal species that has been shown to exhibit an in vitro metabolism profile similar to that of humans. There are two other reasons for determining the metabolite profile prior to the beginning of the Phase II efficacy trials in patients. The first is to detect active metabolites, which might in some cases account for the majority of therapeutic effect, as for instance with losartan (Ohtawa et al., 1993). The second is to determine whether a P450-dependent pathway is responsible for a major part of the elimination of the drug. In the latter case, we may be able to dismiss an entire category of potential drug-drug interactions if most of the drug elimination is not P450-dependent, since concurrently administered P450 inhibitors such as ketoconazole or quinidine (Rendic and Di Carlo, 1997) cannot elevate blood levels of the drug being investigated. Of course, the converse can still be true if the new drug is itself a potent P450 inhibitor. Thus this stage is important to assess the relevancy of prior in vitro metabolic studies, which can only indicate the potential for a P450-dependent metabolism or inhibition.

At the end of clinical trials, then, ideally we will know the metabolic pathways, blood levels, and PK of circulating metabolites, the existence of potentially reactive metabolites, comparative metabolism profiles across several species, and the P450 profile as a substrate, an inhibitor, and an inducer. These data will allow us to understand, and even predict, the PK, pharmacology, and toxicology of the new drug entity under a variety of potential situations, ranging from idiosyncratic individuals and special populations to polypharmacy and some disease states. This is a wealth of clinically useful information, representing the state-of-the-art in DM knowledge, but are there additional things we would like to know? This question serves as one basis for the extrapolation of future trends in DM and will be elaborated upon in a later section.

**Historical Perspective.** Another way to project trends in DM is to look at the forces that have driven DM evolution in the past. The history of DM in industrial drug discovery/development can be characterized as the pursuit of an increasingly greater scientific understanding of the DM aspects of the clinical behavior of new drug entities and correspondingly smaller fractions of purely phenomenological descriptions of the drug. I have, somewhat arbitrarily, divided this evolution into phases. Let us examine these phases and see where they direct us into the future.

**“Chemistry” phase of industrial DM (1950–1980).** Although DM has existed as a science since the 19th century (Conti and Bickel, 1977), it was not incorporated into the development of new drugs until much later. During this “chemistry” phase, the main DM objective for the registration of a new drug entity was to account for the elimination of drug-related materials from the body. This was primarily accomplished by mass balance studies based on the recovery of radioactivity during prolonged collection periods after dosing with a radiolabeled drug. Isolation and identification of metabolites from urine was accomplished to the extent possible. In the earlier part of this period, this literally involved chemical isolation and crystallization of the metabolites, followed by traditional chemical identification methods (i.e., elemental analysis, solid derivatives, infrared spectroscopy, and mass spectrometry). Proof-of-structure was accomplished by unambiguous chemical synthesis of the putative metabolite structure. Obviously, only the major metabolites excreted in the urine were amenable to these classic chemical-identification methods. In the latter part of this period, the introduction of high-performance liquid chromatography made possible the detection of less prominent urinary metabolites as well as any major circulating metabolites. Overall, this phase was largely descriptive, with some understanding of the purely chemical phenomena resulting in a particular metabolic pathway, but little understanding of the biological determinants of the processes, such as the characteristics, regulation, and even location of the enzymes involved.

**“Biochemistry” phase of industrial DM (1975–1995).** In this discussion, the term “biochemistry” is used in a broad sense, encompassing not only the subcellular processes responsible for xenobiotic removal but also the structural chemistry of the enzymes and the molecular biology of the genes involved. This phase began with a decade of basic biochemical research in which the DM enzymes were studied at the molecular level. Proof of the “isozyme” hypothesis of P450 substrate selection was accomplished by the isolation and rigorous biochemical characterization of the hypothesized enzyme forms, thereby demonstrating their actual existence. Subsequent work showed the presence of many more forms of P450 than anyone had guessed existed, based merely on differential substrate metabolism patterns. By the early 1990s, the human P450 enzyme family was
well-established, and it was possible to dissect such phenomena as species differences, interindividual differences, differential enzyme induction, and drug-drug metabolic interactions into the individual contributions made by discrete P450 enzymes, thereby allowing not only understanding of these phenomena in the clinical setting but also their preclinical prediction.

In addition, during this phase we gained an appreciation and understanding of the role of reactive metabolites in certain drug toxicities. In many cases, the reactive metabolites have been found to be the result of P450-dependent oxidations (Nelson, 1995), but nonoxidative processes such as the production of reactive acyl glucuronides have also been recognized (Spahn-Langguth and Benet, 1992). In fact, checking for acyl glucuronides and consequent irreversible plasma protein binding has become a routine part of the DM profiling for carboxylic acid drugs. Also during this phase, we developed a comprehension of the importance of drug transporters in the intestine, liver, and kidney in determining the rate and extent of metabolism (Watkins, 1997).

“Genetics” phase of industrial DM (1990-present and into the future). Today we are seeing the application of the previous decade’s basic research into industrial and clinical practice. We now routinely consider the genetic determinants of DM at the clinical stage and even the discovery stage. This consideration is still limited to phenotyping subjects in clinical trials as “poor” or “extensive” metabolizers of standard substrates for the polymorphic P450 enzymes (i.e. CYP 2C9, 2C19, 2D6) when drug candidates have been found to be predominantly metabolized by these enzymes. We will soon see the genotyping of subjects in clinical trials and, eventually, of all patients. As more information comes in from basic research, we will undoubtedly find polymorphisms in other drug-metabolizing enzymes and drug transporters and will wish to add these forms to the list of genes that are typed. “Gene-chip” technology will soon make large-scale genotyping of many enzymes and proteins a practical reality. This ability to determine the genotype must be accompanied by an understanding of the clinical consequence of the particular polymorph for a particular drug, in the same way that we presently associate CYP 2D6-deficient patients with adverse events with certain drugs. Our ability to manipulate the genes for drug-metabolizing enzymes and drug transporters and will wish to add these forms to the list of genes that are typed. “Gene-chip” technology will soon make large-scale genotyping of many enzymes and proteins a practical reality. This ability to determine the genotype must be accompanied by an understanding of the clinical consequence of the particular polymorph for a particular drug, in the same way that we presently associate CYP 2D6-deficient patients with adverse events with certain drugs.

“Biology” phase of DM (2010?). To extrapolate beyond a few years into the future requires an understanding of the direction of DM research and subsequent application. Toward that end, three premises are introduced here.

I. The driving force for the evolution of DM research has been, and will remain, the need to assure safety and efficacy in the clinical application of new drug entities.

II. Several “black box” areas exist in our current understanding of DM.

III. Therapeutics will see a gradual decline in the use of small organic molecules in favor of peptides, proteins, nucleic acids and viral vectors to manipulate genes to restore normal physiology in diseased tissue.

Premise I should not be very controversial. Although basic knowledge has always been and should continue to be a worthy end unto itself, the majority of funding for DM research by both government and industrial sponsors has been to support the clinical utility of drugs. However, the second two premises might not be as widely accepted.

Concerning Premise II, “black box” problems are those for which we have only phenomological data without a fundamental understanding of the processes underlying the observable phenomena. The following example illustrates what this means: In 1968, Lu and Coon (1968) demonstrated the solubilization of active liver microsomal cytochrome P450, showing that this enzyme was a discrete molecular entity whose activity was not intrinsically linked to its membrane localization. Until then, we had only vague ideas about how an “enzyme” could metabolize so many different substrates. Heme was certainly present in P450, but was P450 a normal protein-based enzyme, or was its activity due to some unique configuration of heme, lipid, protein, and drug? How did it accomplish the unusual reaction of splitting molecular oxygen, putting one of the oxygen atoms into the substrate and reducing the other to water? Why did certain inducers enhance one kind of reaction and other inducers a different one? Lu and Coon showed that the system could be dissected and analyzed. This feat was followed by partial (van der Hoeven and Coon, 1974) and full purification (van der Hoeven et al., 1974; Imai and Sato, 1974), turning a “black box” problem (“What is P450 and how does it work?”) into a reproducible biochemical entity that could be studied and understood by the methods of biochemistry. Soon it was clear that multiple P450 enzymes existed with overlapping substrate selectivities. Subsequently, Dr. Lu and others followed up his basic science contribution by introducing P450-based in vitro methods into industrial research, leading the way to a much better understanding of the metabolism of many real drugs. Today, 30 years after the initial opening of the P450 “black box,” we enjoy such a good understanding of P450 phenomena that characterization of the P450 profile of a new drug has become a routine expectation of the Food and Drug Administration for a new drug filing. And at least nine companies exist partly or mainly by providing P450 products and services.

So, with this example in mind, we can ask the question, What are the remaining “black boxes” of drug metabolism? This is surely where future research will go. It is not hard to list several potential “black boxes” and to imagine the utility that opening them would have for the science of DM. For some of these, we can already see into the partially opened box, while for others we are still speculating:

1. What is the molecular basis for the seemingly mutually exclusive phenomena of high discrimination for oxidation at a particular site on a given substrate and low discrimination among many distinctly different substrate chemotypes by P450 enzymes? This is especially true of CYP 3A4, which also exhibits some poorly understood interactions between substrates and inhibitors (Wang et al., 1997).

2. What are the three-dimensional structures of the DM enzymes, including the P450s, the flavin-containing monoxygenases, the many types of conjugating transferases, the keto-reductases, the carboxylesterases, etc.? Although homology modeling methods are revealing much useful information (Graham-Lorence et al., 1995; De Groot and Vermeulen, 1997; Lewis and Lake, 1997; Szklarz and Halpert, 1997; Tan et al., 1997), we realize that direct structural determination would be much better.

3. How is the reactive oxygen intermediate of P450 produced, what is its structure, and how does it oxidize substrates? Despite over 20 years of effort, the reactive oxygen intermediate has not been isolated and structurally characterized. Recent new proposals for the structure show that although there is a widespread perception that this issue is settled, it remains a subject of debate and surprises (Newcomb et al., 1995; Benson et al., 1997; Vaz et al., 1998).

4. Can we reliably predict the human P450 induction pattern of a new
drug candidate on the basis of preclinical data? There is general acknowledgment that induction in animals can be very misleading, and much better methods, perhaps based on cultured hepatocytes, are needed.

5. How can one predict reactive metabolite-mediated toxicities? This remains possibly the greatest challenge in contemporary DM because it involves the prediction of not only the formation of reactive metabolites but also which cellular nucleophiles are susceptible to attack and what the consequence will be of covalent modification of the nucleophile. In addition, the possibility of free-radical chain reaction initiation by the reactive metabolites is only beginning to be appreciated (Nelson, 1995).

6. What other forms of mammalian P450 have yet to be discovered, where are they localized, and what are their functions? The vast majority of P450 research has focused on the hepatic enzymes. Not until the early 1990s was the importance of intestinal CYP 3A4 realized, and we know now that P450 enzymes can be found in such diverse tissues as skin, nasal mucosa, and brain. What are all these P450 enzymes really for?

Finally, Premise III is the reason for calling this the “biology” phase, referring to the trend toward adjusting the health of diseased cells and tissues by manipulation purely at the biological level. By the middle of the 21st century, therapy will become dominated by the phase, referring to the trend toward adjusting the health of diseased organs, to grow new organs, with the initial culture being in vitro and incorporating engineered genetic material to prevent recurrence of the original disease state. At some point, we will seek to prevent disease rather than cure it. This will become possible with the elucidation of the sequence of the complete human genome, expected to be complete within a few years, and subsequent comprehension of the information therein. Anticipation and prepathological correction of potential diseases (e.g., breast cancer) will first be accomplished with adults but will inevitably regress to earlier stages (adolescents, children, neonates, fetuses, eggs, and sperm). We must strive to ensure that society develops a humane and rational policy about such eugenic programs, preserving and encouraging the diversity of the human genome, but the imperative to ease human suffering will, without a doubt, bring such capabilities.

The main consequence of this trend to manipulate the biology at a fundamental level will be accompanied by a gradual decline in our reliance on small molecules as therapeutic agents (fundamental level will be accompanied by a gradual decline in our reliance on small molecules as therapeutic agents, and the imperative to ease human suffering will, without a doubt, bring such capabilities. Finally, Premise III is the reason for calling this the “biology” phase, referring to the trend toward adjusting the health of diseased cells and tissues by manipulation purely at the biological level. By the middle of the 21st century, therapy will become dominated by the phase, referring to the trend toward adjusting the health of diseased organs, to grow new organs, with the initial culture being in vitro and incorporating engineered genetic material to prevent recurrence of the original disease state. At some point, we will seek to prevent disease rather than cure it. This will become possible with the elucidation of the sequence of the complete human genome, expected to be complete within a few years, and subsequent comprehension of the information therein. Anticipation and prepathological correction of potential diseases (e.g., breast cancer) will first be accomplished with adults but will inevitably regress to earlier stages (adolescents, children, neonates, fetuses, eggs, and sperm). We must strive to ensure that society develops a humane and rational policy about such eugenic programs, preserving and encouraging the diversity of the human genome, but the imperative to ease human suffering will, without a doubt, bring such capabilities.

The main consequence of this trend to manipulate the biology at a fundamental level will be accompanied by a gradual decline in our reliance on small molecules as therapeutic agents (i.e., drugs as we commonly think of them) and the introduction of gene repair/replacement modalities as first-line therapy. One may ask, “Is disease one thing, but what about accidents or infections?” However, in the future, physicians will be able to induce rapid tissue regeneration to treat physical trauma such as cuts, hemorrhages, or broken bones. The risk of infection will be low, because people will be equipped from birth with a complete set of robust immune responses to all known pathogens. On the other hand, even biology doesn’t use macromolecules for everything; normal biochemistry involves many small organic molecules as hormones, messengers, cofactors, etc. (e.g., steroids, prosta-

References


