Centennial Perspective

The Development of Drug Metabolism Research as Expressed in the Publications of ASPET: Part 1, 1909–1958

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Received June 6, 2007; accepted July 30, 2007

ABSTRACT:

This is the first of three articles covering the development of drug metabolism research in the United States during the first 100 years of the American Society for Pharmacology and Experimental Therapeutics (ASPET). Before 1909, the majority of drug metabolism research was performed in Europe. The period from 1909 to 1958 saw extensive development of the methods required for modern metabolism studies. Examples of trends and specific discoveries are drawn from the archives of ASPET publications.

The history of science is one of individual discovery followed by reproduction, verification, expansion of scope, extended applications, and, ultimately, establishment of principles that can be used on a much broader basis. These findings and theories are recorded in the literature for use by scientists throughout the world. Scientific societies, as exemplified by the American Society for Pharmacology and Experimental Therapeutics (ASPET), have taken the responsibility of producing publications and scientific forums that maintain the highest standards and greatest diversity of thought within their prescribed disciplines. At the time of the inception of ASPET, the greatest challenge was dissemination of scientific findings, whereas, at our 100th anniversary, the major challenges include ensuring the integrity of the science and providing the most appropriate media for communication. Throughout the 100-year history, those unsung heroes, the reviewers and editors, have provided the underpinnings for a truly remarkable record of publications.

The development of drug metabolism in the United States is well represented in the pages of the ASPET journals. This review will concentrate on the progress of the field in the last 100 years as chronicled mainly by those publications. A similar history could be elucidated from other journals, especially the Journal of Biological Chemistry (JBC), but the purpose of this review is to herald the 100th anniversary of ASPET, so some introspection should be forgiven. Although we are concentrating on the domestic society, we also recognize the powerful influence of the worldwide scientific community. Other local histories have been written and provide excellent perspective relative to the U.S. experience (Parke, 1997; Kato and Yamazoe, 2000). In addition, a number of global histories are available (Conti and Bickel, 1977; Bachmann and Bickel, 1985; Murphy, 2001). In 1908, the developing science of pharmacology was one of observation and experimentation. The main goal was to determine the effect of compounds on living organisms and to record the results. It was not within the capabilities of the science of that era to understand how a compound was causing its effect. Through the century, this science of observation grew to explore the cause and effect, in isolated tissues and organs, at the enzyme level and at the molecular level. To relate cause to effect necessitated an estimate of actual drug exposure. In many cases, the discrepancies between in vitro and in vivo studies also pointed to the need to understand the fate of compounds in the body. Determining the rate and extent of absorption, metabolism, distribution, and elimination of investigative molecules gradually became part of the overall pharmacological evaluation.

At the dawn of the 20th century, metabolism research had been taking place in Europe for over 50 years (Murphy, 2001). Wohler had delineated the formation of hippuric acid from benzoic acid in humans. Oxidation had been discovered by Schultz and Naunyn in the conversion of benzene to phenol. Reduction, methylation, acetylation, sulfation, and glucuronidation had all been elucidated by the chemists of Europe, especially Germany (Murphy, 2001). Many of the founders of ASPET had their early training in the labs of these German pioneers. Nonetheless, when John J. Abel invited 18 pharmacologists to Baltimore in 1908 with the purpose of forming a new society, there was only marginal interest in drug metabolism. The desired name, the American Pharmacological Society, was changed at the last minute to include Experimental Therapeutics (Chen, 1969). In retrospect, it was that inclusion that encompassed the breadth of drug metabolism research.

In that same year, Paul Ehrlich received the Nobel Prize for Medicine for his work on understanding the immune response. Ehrlich’s theory of “magic bullets,” or antibodies in search of toxins, was later expanded to encompass attempts at drug therapy. Moving from the immune system to chemotherapy, Ehrlich’s efforts in understanding the chemistry of the arsenical atoxyl led to the discovery of arsphenamine, the first effective treatment for syphilis. The story of arsphenamine illustrates the value of both pharmacology and drug
metabolism. Ehrlich established a system of testing that rivals the modern day pharmaceutical approach to development of new agents. He established both in vitro and in vivo assays for assessing the effects of new compounds on the viability of spirochetes. Ehrlich’s coworker, Sahachiro Hata, discovered the antisyphtilic activity of molecule 606, arsphenamine, when he successfully treated infected rabbits (Ehrlich and Hata, 1911). The compound had no significant activity on the spirochetes in vitro even though it had high activity in vivo. The in vivo activity was later explained by Voegtlin and Smith when they determined that arsphenamine is actually a prodrug that is oxidized to the active arsenoxide (Voegtlin and Smith, 1920). There have been 98 publications on studies related to arsphenamine in the Journal of Pharmacology and Experimental Therapeutics (JPET). Many of these dealt with understanding why this potent arsenical is more toxic to the spirochete than to human cells. Ehrlich hypothesized that the compound worked through interaction with sulfhydryl groups in the spirochete. Voegtlin et al. (1925) expanded on this hypothesis by showing that glutathione could have a sparing effect on the toxicity of arsenicals. He proposed that glutathione might be the receptor postulated by Ehrlich.

Sixty years later (Fairlamb et al., 1985), it was discovered that trypanosomes have a unique sulfhydryl agent, trypanothione, which is probably the main target of the metabolically formed arsenoxide. Interestingly enough, a revised structure of arsphenamine was published in 2005 (Lloyd et al., 2005). The story of arsphenamine is the initiating story of modern chemotherapy, and the 100 years of ASPECT parallel this developing field. Pharmacology and experimental therapeutics are intertwined, and drug metabolism is a vital part of that marriage.

In an address to the Michigan Pharmaceutical Association in 1891, Abel described pharmacology as follows: “Briefly, this science tries to discover all the chemical and physical changes that go on in a living thing that has absorbed a substance capable of producing such changes, and it also attempts to discover the fate of the substance incorporated” (Parascandola, 1992). Abel’s interest in the fate of administered compounds is illustrated in his first publication in the new journal JPET (Abel and Rowntree, 1909). In this study, Abel and Rowntree examined the effects of a series of substituted phthalimides. With the goal of finding a useful injectable purgative, they examined the compounds in dogs with emphasis on the excretion, reabsorption, and purgative action. Using a basic solution to reveal the brightly colored phthalimides, the authors managed to show excretion by the liver and reabsorption of the substances from the bile during its passage through the large intestine.

These studies illustrated an important aspect of disposition studies in this time period—the need for readily measured properties. Using highly colored substances to illustrate physiological principles was the rule of the day. Indeed, Ehrlich’s development of the antisyphtilic drug arsphenamine evolved from the use of dyes to stain membranes to the thought that the specific binding observed with the dyes could be adapted to provide the “magic bullet” needed to attack the damaging organism. Ehrlich’s structure activity studies focused on modification of dye molecules, and this also was the starting point for Domagk’s later work on the sulfanilamides.

Additional illustrations of the methodology of the early 20th century come from two other papers in the first volume of JPET. Toral Sollman, Paul Hanzlik, and J. Douglas Pilcher (Sollmann et al., 1910) studied the quantitative effects of phenol administration. They were studying the fact that phenol seemed to inhibit the absorption of itself as well as other drugs. One of the reasons that they chose phenol was because of the “relative ease and accuracy with which phenol can be quantitatively determined.” This “easy” method involved grinding of the tissue, steam distillation of the mash, reaction of the phenol containing solution with Br₂, titration of the remaining Br₂ with KI, and determination of the amount of iodine produced with Na₂S₂O₃ titration (Sollmann et al., 1910). An alternative to colorimetric assays was the use of a bioassay to follow the active compound. Thus, a study on the “fate of strychnine in the body” (Hatcher and Eggleston, 1917) used strychnine’s unique toxicity to follow unchanged drug. A bioassay of strychnine using its effects on the fasting tree frog allowed the authors to follow the levels of strychnine-like activity in the cat.

Given the limits of the analytical methodology, there were few publications in the early years of JPET that actually dealt with metabolic transformation. Probably the biggest boost to the interest in drug metabolism came with the discovery of the sulfanilamide antibacterials. Taking Ehrlich’s lead, Heinrich Horlein, at the largest chemical company in the world, IG Farben, assembled a team of chemists, bacteriologists, and pharmacologists to develop new drugs starting from the many dye nuclei that were available. In 1927, he hired Gerhard Domagk to expand the laboratory efforts. Domagk, together with chemists Josef Klarer and Franz Mietzsch, prepared and tested hundreds of compounds in infected animals. After 5 years and over 700 compounds, Domagk found an agent effective against bacteria in his animals. The compound, to be named prontosil, was a red azo dye derivative containing a sulfonamide side chain. The fact that it had little antibacterial activity in vitro was troubling, but IG Farben proceeded to the market. In 1935, they began to publicize the effects of prontosil.

In the UK, Leonard Colebrook obtained a sample of prontosil and began a series of studies that led to a clinical trial in 1936. Colebrook and Kenny’s publication in 1936 (Colebrook and Kenny, 1936) cemented the reputation of prontosil and stimulated efforts throughout the world. In France, in the laboratory of Ernest Forneau, prontosil was tested, along with a number of other azo dyes, as was sulfanilamide on its own (Trefoulet et al., 1935). Surprisingly, the sulfanilamide worked just as well as prontosil. They immediately postulated that prontosil was, in fact, a produg and the active principle was sulfanilamide. This stunning result had two major effects: it launched a sulfanilamide frenzy where all major pharmaceutical companies began making derivatives, and it made the world take notice of the body’s ability to convert inactive substances to active molecules via metabolism. Although arsphenamine had been the initial chemotherapeutic example of drug activation, the sulfanilamides had much greater impact due to their widespread use. In the period from 1936 to 1941, there were 50 publications in JPET on the sulfanilamides.

One of the pioneers of this period was E. K. Marshall. Marshall’s career in drug metabolism has been well documented by M. Bickel (1996). He was a student, collaborator, and eventually successor to John Abel at Johns Hopkins University. He was editor of JPET from 1932 to 1938 and president of the Society in 1942. Among his many scientific contributions, Marshall’s studies on the sulfonamides stand out for their groundbreaking methodology. He developed a method of analysis with collaborator Bratton (Bratton and Marshall, 1939) that was the standard analysis of sulfonamides for 40 years. Armed with a sensitive assay, he elaborated the relationships between blood levels, tissue levels, and therapeutic responses. His studies on the distribution of sulfanilamide (Marshall et al., 1937) set the standard for all future distribution studies. Marshall also played a major role in the government antimalarial program launched shortly after the start of World War II. It was Marshall who recruited James Shannon to that program, and it was Shannon who was responsible for assembling a task force including Bernard Brodie, Sidney Udenfriend, and others. Using methodology developed by Brodie and Udenfriend, Shannon and coworkers established an understanding of the fate of the antimalarial
quinacrine hydrochloride (Atabrine; Abbott Laboratories, Abbott Park, IL) that led to its effective use in our troops throughout the war. Their JPET article published in 1944 clearly outlines their rationale (Shannon et al., 1944). The new dosing regimen, based on the pharmacokinetics of the compound, was more effective and had fewer side effects than earlier dosing schedules. The acceptance of the compound was crucial for soldiers in World War II for the treatment and prevention of malaria.

The interest in sulfonamides also sparked studies on the mechanism of metabolism of the various derivatives. The first in vitro study of the reduction of the azo bond of neoprontosil was by Bernheim in 1941 (Bernheim, 1941). This was one in a series of pioneering in vitro studies from the laboratory of the Bernheims at Duke University. Studies on the hydrolysis of acetanilide (Michel et al., 1937), conjugation of phenol (Bernheim and Bernheim, 1943), conjugation of morphine (Bernheim and Bernheim, 1945a), and hydrolysis of meperidine hydrochloride (Demerol; Sanofi-Synthelabo, New York, NY) (Bernheim and Bernheim, 1945b) all led to the conclusion that the liver was the main site of metabolism of these agents.

A secondary aspect of the sulfanilamide boom was a tragedy that ultimately led to the expansion of the Food and Drug Administration (FDA). In a rush to market an elixir formulation of sulfanilamide, a chemist at the S. E. Massengill Company prepared a solution containing diethylene glycol. The formulation caused over 100 deaths before the majority of the preparation was removed from pharmacy shelves. The uproar over this tragedy directly led to the new Federal Food, Drug and Cosmetic Act, which was passed by Congress on June 2, 1938. This Act required that new drugs be proven safe before marketing, all ingredients be identified, and recommendations for use be included in the package. The legislation changed the pharmaceutical industry in a most dramatic fashion. A number of studies on diethylene glycol and other glycols were published in JPET, including a study from the newly formed division of pharmacology at the FDA (Morris et al., 1942).

An excellent illustration of the challenges presented by the need for sensitive analytical methods is the study of acetanilide metabolism. When Lester and Greenberg studied the fate of acetanilide, they specifically looked for aniline and, failing to find measurable amounts, concluded that aniline was probably not involved in the action of this drug (Greenberg and Lester, 1946; Lester et al., 1947). However, Axelrod and Brodie used the recently developed counter current distribution techniques of Craig (Craig, 1944) and the extraction methods published by Brodie and coworkers (Brodie et al., 1945; Brodie et al., 1947) to develop a method for aniline that was more than 10-fold more sensitive than that used previously (Brodie and Axelrod, 1948a). With the higher sensitivity, they were able to show that the levels of aniline corresponded to the methemoglobin formation (Brodie and Axelrod, 1948b). In addition to identifying the toxic principle, they also showed that a hydroxylated metabolite was the main metabolic product. This compound, later marketed as Tylenol, was shown to be a potent analgesic.

The 1948 publication on acetanilide was also a landmark for another reason. It was the first foray of Julius Axelrod into the world of metabolism. Before this work, Axelrod had spent 11 years in the Laboratory for Industrial Hygiene in New York. By his own recollections, he was content in this job and planned to stay. However, a health crisis with the analgesic acetanilide led him to the laboratory of Bernard Brodie. This was the start of a collaboration that would launch the career of the eventual Nobel Prize winner Axelrod.

Brodie was rapidly developing his reputation in the field through the publication of groundbreaking papers on the analytical methodology necessary for determining levels of drugs and their metabolites. Brodie’s laboratory at the National Institutes of Health became a mecca for scientists interested in the study of the disposition of drugs (Costa et al., 1989). Over 100 of Brodie’s approximately 400 publications are found in JPET. They tell the story of an illustrious career and a group of collaborators unsurpassed in the field.

In this same time period, a major event in the field took place when R. T. Williams published the first edition of Detoxication Mechanisms in 1947. This book, and its subsequent expansion in 1959, established the field of “drug” or “xenobiotic” metabolism as a discreet field of study. The books are remarkable in their scope, organization, and completeness. Organized by chemical groups, Williams explored the variety of reactions that had been delineated and their cross-species comparisons. Williams wrestled with the title, which had been favored by his publishers but clearly did not accurately represent the content of the volumes. The subtitle of the second edition was Williams’ choice as the most representative title: The Metabolism and Detoxication of Drugs, Toxic Substances, and Other Organic Compounds.

The problem was that the activities of the metabolic products had more to do with the nature of the precursor than with the ability of the enzymatic catalysts. Although many compounds are “detoxified,” many others are activated and/or made more toxic. Williams accurately summarized the state of knowledge in 1959 (and still today) in the following passage:

It is clear that, from the point of view of detoxication, phase I reactions cannot be considered as detoxication mechanisms although in many cases detoxification does occur as a result of these reactions. Phase II reactions on the other hand seem to be largely processes of detoxication but again exceptions occur. It is therefore very difficult to decide to what extent a systematic true detoxication occurs in the body. Detoxication nevertheless occurs, but with an entirely foreign compound it is largely a matter of chance whether it takes place efficiently enough to protect the organism completely from the noxious effects of the foreign compound. (Williams, 1959, p. 739)

The publication of William’s initial monograph represents the birth of the field of drug metabolism, and the next 12 years are probably the time of its most rapid growth. This culminated in the second volume of Detoxication Mechanisms, a greatly expanded version of the original. The first edition had 861 references, whereas the second had 2372. Over 23% of the new references came from JPET (12.8%) and JBC (11.0%). This is reflective of the rapid expansion of research in the United States. From 1900 to 1905, 63% of the publications in drug metabolism originated in Germany, and 92% were written in German. From 1940 to 1947, 84% of the papers were written in English, and the focus was centered on the United States (Bachmann and Bickel, 1985).

With the rapid rise of pharmacology in the postwar United States, the board of ASPET decided to launch Pharmacological Reviews to publish articles summarizing the progress in the broad areas of research encompassed by pharmacology. The first issue was published...
in 1949,3 A review of the metabolism of barbiturates in the first volume is an excellent example of the quality of the articles and provides a nice overview of the methods of analysis available at the time (Maynert and Dyke, 1949). This era saw new technology that had been developed during the war come into use in the research lab. This included the use of radioisotopes and high-speed centrifugation. In addition, the development of partition chromatography by Martin and Synge heralded the addition of new separation tools including paper, thin layer, and gas chromatography. The first 14C study published in JPET was by Elliott and coworkers in collaboration with the radiation laboratory at Berkeley (Elliott et al., 1949). They looked at the distribution of 14C methadone in the rat. This powerful analytical tool went on to be a staple of drug disposition studies.

There was an increased emphasis on the quantitative evaluation of the disposition of new and old drugs. One of the most prominent authors in this time period was Thomas Butler.4 Butler was impressed by E. K. Marshall’s approach to drug metabolism and made a number of quantitative studies on drugs such as the barbiturates, diphenylhydantoin (Butler, 1957), trimethadione (Butler, 1953), phe- nobarbital (Butler et al., 1954; Butler, 1956), and the anesthetic chloride hydrate (Butler, 1948).

This period also contained some of the initial publications of Carl Smith from Christ Hospital Institute in Cincinnati (Schmidt et al., 1947; Smith, 1956). In addition to his many JPET publications, Smith made a major contribution to the Society as founder and editor of the Drug Metabolism Newsletter, a publication for Society members interested in the events of the drug metabolism community.

Studies on the conversion of codeine to morphine provide a picture of the developing drug metabolism science. In 1941, Oberst looked for the presence of morphine in the urine of codeine-treated addicts and failed to find significant quantities (Oberst, 1941). When the Bernhems studied codeine metabolism in vitro, they found a substance reacting like morphine, but they could not draw the conclusion that it was in fact morphine (Bernheim and Bernheim, 1944). Latham and Elliott used radiolabeled codeine to show demethylation in the rat in vivo (Latham and Elliott, 1951). Furthermore, Adler and Shaw were able to identify the morphine formed in rat liver slice incubations of codeine (Adler and Shaw, 1952). Mannerings5 used paper chromatography to separate the morphine formed in humans after ingestion of codeine (Mannering et al., 1954). Axelrod followed these studies using his newly discovered microsomal system to show that codeine was metabolized by rabbit liver microsomes to morphine (Axelrod, 1955a). Thus, in a period of less than 15 years, the field went from the thought that morphine was not involved in the action of codeine to an initial understanding of the enzymes involved in that important conversion.

In the late 40s and early 50s, there was a major movement to biochemical approaches to drug metabolism. The structures of the cofactors nicotinamide adenine dinucleotide phosphate, S-adenosyl methionine, coenzyme A, and phosphoadenosine phosphosulfate were all elucidated in the 50s. The discovery of a unique inhibitor of the actions of hexobarbital (Cook et al., 1954b) and a variety of other compounds (Cook et al., 1954a; Swinyard et al., 1954) at SKF led to the examination of the compound SKF 525a in Brodie’s laboratory. When Axelrod discovered the microsomal activity involved in amphetamine deaminase (Axelrod, 1954), the inhibitor studies were expanded to include this system. The resultant publications on the action of SKF 525a (Axelrod et al., 1954; Cooper et al., 1954) and the properties of the microsomal oxidase system that required oxygen and NADPH (Axelrod, 1955a;b; Brodie et al., 1955; Cooper and Brodie, 1955) launched a new era in drug metabolism.

By 1958, the stage was set for the discovery of cytochrome P450. Gillette and coworkers discussed the potential mechanisms of the microsomal oxidases and speculated on the similarity of the drug oxidations and steroid hydroxylases (Gillette et al., 1957).6 Klingenberg (1958) and Garfinkel (1958) had observed a red pigment in microsomes of unknown nature and function. The second 50 years of ASPET will see the denouement of this great riddle.

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