British Journal of Pharmacology (2006) 147, S89–S99
www.nature.com/bjp

Drug metabolism and pharmacogenetics: the British contribution to fields of international significance

***John Caldwell**

Faculty of Medicine, The University of Liverpool, Duncan Building, Daulby Street, Liverpool L69 3GA

The branch of pharmacology we now call 'drug metabolism', the consideration of the enzymes and procesess determining the disposition of drugs in the body, emerged in the 1840s on the continent of Europe, but British science made little or no contribution until the 1920s. From this point on, the development of the field through the 20th century was shaped to a very significant extent by a series of influential British workers, whose contributions were of global significance and who can now be seen as fathers of the subject. Since the 1950s, and gaining pace inexorably from the 1970s, the significance of drug metabolism to human therapeutics has been greatly added to by the emergence of pharmacogenetics, clinically important hereditary variation in response to drugs, which underpins the current emphasis on personalised medicine. This review examines the British contributions to both these fields through the lives of seven key contributors and attempts to place their work both in the context of its time and its lasting influence.
British Journal of Pharmacology (2006) **147,** S89–S99. doi:10.1038/sj.bjp.0706466

Keywords: Drug metabolism; pharmacogenetics; Boyland; Dutton; Garrod; Gowland Hopkins; Price Evans; Smith; Williams; glucuronic acid conjugation; glutathione conjugation; acetylation polymorphism; CYP2D6 polymorphism

Abbreviations: CYP, cytochrome *P*450; FMO, microsomal flavin-containing mono-oxygenase; ICI, Imperial Chemical Industries; NMR, nuclear magnetic resonance; TMA, trimethylamine; TMAO, trimethylamine N-oxide; UDP, uridine diphosphate; UDPGA, uridine 5′-diphosphoglucuronic acid

Introduction

The term 'drug metabolism' has entered the popular lexicon as a shorthand term for the biochemistry of foreign compounds, that is the science describing the absorption, distribution, metabolism and elimination of substances foreign to the energy-yielding metabolism of the organism. This field of endeavour is now some 165 years old, the first report of the fate of an exogenously administered compound appearing in 1840. The work covered by this area has changed constantly over time. The first 60 or so years, up to 1900, represent a time when the principal features of the fate of foreign compounds were discovered. These include the main metabolic pathways as well as the principal routes of elimination, together with the elucidation of a few physiological factors influencing these. At this early stage, there was little distinction between these studies and the biochemistry of endogenous compounds, if only because of the (relative) ease of analysis of foreign compounds. As the chemical repertoire of metabolic pathways was increasingly defined, the biological context began to emerge. Work with perfused organ preparations showed that the liver was the principal site of metabolism, with contributions from the kidney and other organs also being evident. Some of the most noted early pharmacologists contributed to these studies, including Rudolf Buchheim, Bernhard Naunyn and Oswald Schmiedeberg, and increasingly they began to consider the biological consequences of metabolism. The initial view was that these processes represented detoxications, protecting the body against foreign chemicals, but this view has been successively refined with the passage of time. Some of the earliest work on the pharmacological importance of metabolism came from work in the 1930s on the first sulphonamides, which were activated by azo reduction. The appreciation that their activity was mediated by a metabolite resulted in the rapid emergence of more effective drugs. Many more examples of pharmacologically active metabolites have accrued over the intervening years.

The growth of this field has, like all science, been driven by the emergence of new technologies, which in turn enable new problems to be addressed. After the Second World War, developments in drug metabolism came from major advances in bioanalysis, which progressively developed from simple colorimetry through to today's sophisticated hyphenated techniques, linking advanced separations with mass spectrometry and NMR as detection systems. At the same time, new biological techniques emerged, allowing the enzymic basis of these reactions to be explored. These systems identified the hepatic microsomal oxidising system to be responsible for the metabolism of a wide variety of drugs and other chemicals. Whole animal studies showed the importance of physiological variables as determinants of metabolism, with demonstrations of the influence of age, sex, nutrition, animal species and the like. One important discovery was the ability of some drugs and other xenobiotics to enhance the metabolism of themselves and others, the phenomenon of 'enzyme induction'.

In the 1970s, the enzymes themselves were separated, confirming suspicions that most existed as families of related isoenzymes and the subsequent rapid emergence of biochemical genetic techniques facilitated the investigation of the

*Author for correspondence; E-mail: jcc@liv.ac.uk

regulation of these enzyme families. These studies link with important discoveries of human genetic polymorphisms of drug metabolism to place this field at the forefront of the emergence of 'personalised medicine' for the 21st century.

The history of these related subjects shows that there has been a distinctive, sustained and substantial British contribution. With one notable exception, this emerges from 1930 onwards.

The beginnings of drug metabolism 1840–1914

It would appear that the first report ever on the transformation of an exogenously administered compound into another metabolite was by Alexander Ure in 1841. In a note entitled 'On Gouty Concretions with a New Method of Treatment' in the London Medical Gazette, Ure reported that benzoic acid was converted by humans to hippuric acid. Alexander Ure was born in Glasgow in 1810, studied at Edinburgh University, and eventually settled in London where he acquired a large medical practice. In 1854, he was among the first consultant staff of the then new St Mary's Hospital in Paddington (of which more later). He was president of the Harveian Society in 1857 and a member of the Pharmaceutical Society.

Ure had a particular interest in the treatment of gout, perhaps related to the fact that his father Andrew, a renowned scientist, suffered from this debilitating disease. He was apparently familiar with the proposal of Wöhler that benzoic acid could be converted to hippuric acid in the body. He deduced that this process might utilise urea and thereby diminish the symptoms of gout. Although his work was successful in demonstrating the excretion of hippuric acid following administration of benzoic acid, later studies showed that even in the presence of hippuric acid the level of uric acid in the urine was not diminished.

However, this work was rapidly overtaken by developments in Continental Europe, which initiated the systematic study of the transformations of xenobiotics in the animal body. In 1842, Wilhelm Keller, a pupil of Wöhler and Leibig, confirmed the conversion of benzoic acid to hippuric acid (Keller, 1842). At this time, these studies were part of the emerging realisation that living systems had a chemistry of their own and the leading investigators were those responsible for major advances in what in Germany particularly was known as 'physiological chemistry'. Between 1860 and 1900, the great majority of the metabolic pathways were discovered (see Conti & Bickel, 1977).

Table 1 Discovery of the Major Pathways of Xenobiotic Metabolism in the 19th Century

1842, 1867	Oxidation
1863	Reduction
1875	Reduction
1876	Sulphation
1879	Glucuronic acid conjugation
1879	Mercapturic acid synthesis
1887	Methylation
1893	Acetylation

Building upon this foundation, a distinctively British contribution began to emerge after the First World War. It is not possible in the present context to provide encyclopaedic coverage of this. Rather, it will be considered in terms of four people who in different ways may be regarded as among the grandfathers of the subject and whose recognition and influence were global.

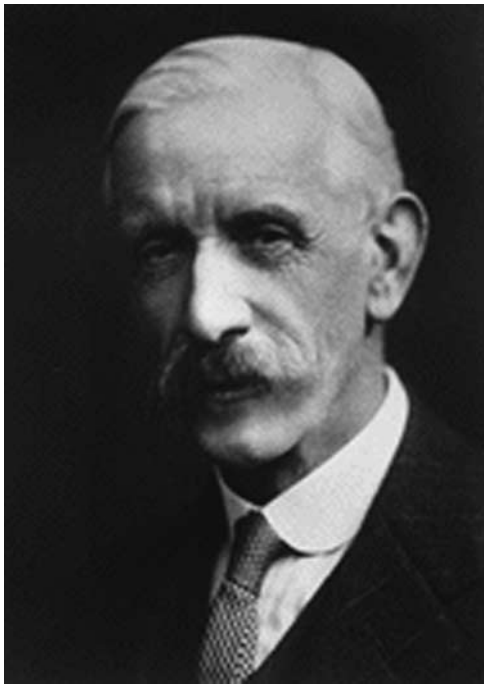

Figure 1 Sir Frederick Gowland Hopkins in 1908.

Sir Frederick Gowland Hopkins, 1861–1947

Gowland Hopkins (Figure 1) shared the 1929 Nobel Prize for Physiology with Christiann Eijkman for the discovery of vitamins and is widely recognised for a series of other contributions including work on the biochemistry of muscle contraction and the discovery of tryptophan.

By today's standards, Gowland Hopkins presents a fine example of a 'nonstandard' career. Leaving the City of London School at 17 he obtained a BSc in chemistry by evening classes in 1885. He then went to Guy's Hospital to read medicine, graduating in 1894, aged 32. During this time he met and forged what was to be a life-long friendship with Archibald Garrod (*vide infra*), which was a major influence on the careers of both men. After 4 years teaching physiology and toxicology at Guy's, he moved to Cambridge in 1898 to develop the new science of physiological chemistry, which he achieved with spectacular success. Setting his slow start behind him, he was elected to the Royal Society in 1905, only 11 years after qualifying in medicine. His career is described in the commemorative volume 'Hopkins and Biochemistry' (Needham & Baldwin, 1949) produced for the 1st International Congress in Cambridge in 1949 and has received a modern critical review by Kamminga & Weatherall (1996) and Weatherall & Kamminga (1996).

Gowland Hopkins was acutely aware of the importance of the German studies on the metabolism of exogenous compounds. His Presidential Address to the British Association in 1913 (Hopkins, 1913), 'The dynamic side of biochemistry' was widely publicised at the time and repays reading now. It has been analysed by Kamminga & Weatherall (1996). Hopkins emphasised that biochemistry dealt with simple substances undergoing comprehensible reactions and stoutly rejected the

old German saw '*Thierchemie ist schmierchemie*' (animal chemistry is greasy chemistry). The great majority of his illustrations were those listed in Table 1, from the work of Ure onwards.

This emphasis on the significance of the metabolism of exogenous compounds was extremely influential. At that time the principal interest in these studies was in terms of the insights they offered into endogenous biochemistry: exogenous moieties such as the largely stable benzene ring served as convenient 'labels' for molecules, much as we employ isotopic labelling today. Thus, ω-phenyl fatty acids with even numbers of carbon atoms are oxidised to phenylacetic acid, while those with odd numbers of carbons yield benzoic acid, findings that underpin the elucidation of the β-oxidation of fatty acids. While future work on intermediary metabolism moved away from these techniques, the early studies provided a firm base for the construction of a science of the metabolism of exogenous compounds.

Aside from the emphasis he placed upon early, largely German, studies of drug metabolism, Gowland Hopkins merits his place in the present context by his discovery of glutathione, which we now understand to play a critical role in cellular responses to many types of toxic insult. In 1921, his paper 'An autoxidisable constituent of the cell' postulated that this entity, termed glutathione, was the dipeptide glutamylcysteine (Hopkins, 1921). Between 1922 and 1930, a series of papers delineated its key roles in cellular biochemistry, which can now been seen as presaging many of the present areas of biochemistry where glutathione is important, notably in redox biochemistry (Hopkins, 1923). The identity of glutathione as the tripeptide γ-glutamylcysteinylglycine was confirmed in 1929.

Nowadays, rather than for his concept of dynamic biochemistry, Gowland Hopkins is principally known for what was a relatively minor sideline in his main research, which came to assume great importance: the discovery of vitamins (Weatherall & Kamminga, 1996). Although from 1930 onwards, the emergence of British drug metabolism passes Gowland Hopkins by, his inclusion here is richly merited by his early recognition of and spokesmanship for the emerging field as well as the discovery of glutathione.

Richard Tecwyn Williams, 1909–1979

Williams (Figure 2) was universally known by his initials 'RT'. Born in Abertillery in South Wales in 1909, he graduated in chemistry in Cardiff in 1929 and then worked for his PhD with John Pryde in the Physiological Institute in Cardiff. His 1932 thesis provided the first full characterisation of glucuronic acid, which he obtained from the glucuronides excreted by dogs given the terpenes camphor and borneol. He moved successively to Birmingham (1934–1942) and Liverpool (1942–1948) before appointment as the first Professor of Biochemistry at St Mary's Hospital Medical School in London. Over a period of more than 40 years, Williams developed the work of his PhD thesis into a broad and systematic consideration of the fate of exogenous chemicals in the animal body. Between 1938 and 1958, he was senior author of 77 papers in the series 'Studies in Detoxication' in the Biochemical Journal, a series brought to a close only by the refusal of the journal to accept numbered series of papers, since so few series went beyond 2!. In addition, he published more than 50 papers on terpenes and from the late 1930s onwards worked extensively on sulphonamides.

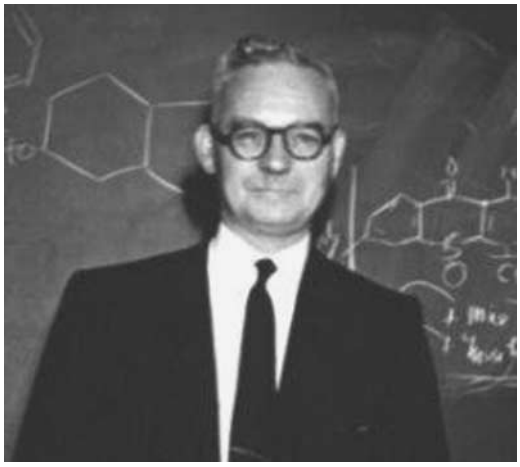

Figure 2 R. Tecwyn Williams in the early 1950s.

Towards the end of the Second World War, Williams started work on the book that was to confirm his place as the most influential scientist of his time in this area, '*Detoxication Mechanisms: The Metabolism of Drugs and Allied Organic Compounds*' (Williams, 1947). His preface to the first edition of states

> In writing this book, my object has been to gather together in orderly fashion the available information on the metabolic fate of organic compounds foreign to the body, so that working hypotheses can be advanced.

The chapter headings he chose confirm the 'orderly fashion' he sought, moving systematically on the basis of chemical structure with increasing complexity of functional groups, substituents and ring structures. The titles were the metabolism of (a) some aliphatic compounds and cyclohexane derivatives; (b) aromatic hydrocarbons, halogenated aromatic hydrocarbons; (c) phenols; (d) aromatic alcohols, ethers, aldehydes, ketones and amides; (e) aromatic acids; (f) organic cyanides; (g) aromatic nitro, amino and azo compounds; (h) sulphones, sulphonic acids and sulphonamides; (i) terpenes and camphors; (j) heterocyclic compounds; and (k) organic compounds of arsenic.

Williams' systematic approach allowed the discernment of basic principles amidst the welter of examples of the various metabolic reactions and he was able to assemble these into metabolic pathways and schemes. His most important work was his realisation that the vast majority of compounds underwent a biphasic metabolic sequence, in which a Phase I reaction of oxidation, reduction or hydrolysis is followed by a Phase 2 conjugation reaction, in which the key functional group (-OH, -NH, -SH) introduced or revealed by the Phase I reaction is linked with a conjugating agent derived from normal cellular biochemistry.

In 1947, issues of the biological activity of the chemicals and interfaces with pharmacology and toxicology were very much secondary considerations for Williams but this changed markedly over the years. Williams was aware that the short title of his book was actually a misnomer, since he was aware of many instances where metabolism actually *increased* toxicity. He stated his feelings clearly in the last paragraph of the Second Edition (Williams, 1959):

> It is clear that, from the point of view of detoxication, phase I reactions cannot be considered as detoxication mechanisms although in many cases detoxication does occur as a result of these reactions. Phase II reactions on the other hand appear 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.

With the emergence of major groups of synthetic drugs from the mid-1950s and his appreciation of developments in North America, notably through his close friendship with Bernard Brodie, Williams' interests extended to take in a series of key issues that can now be seen as important parts of the foundations of modern chemical safety evaluation. A major contribution to raising this field to its present significance came from the work of this laboratory on understanding of thalidomide (see, e.g. Fabro *et al*., 1965). In addition, the group published over 30 papers on species differences in metabolism, which provide a systematic basis for the selection of species for safety testing.

Williams' influence was sustained and extended by the Schools which originated from his influence. In the 1930s, while still a junior Lecturer, he catalysed work in Birmingham by Thorpe and Bray, which continues to the present day (*vide infra* and also see Mitchell & Waring, 1997). Ken Dodgson, a student in Liverpool, moved to Cardiff in 1948 and became Head of Biochemistry in the late 1950s. He oversaw over 30 years of work in this field, with major contributions to sulphation, notably in association with Gillian Powell, who succeeded him as Head of Department. Dennis Parke worked with Williams at St Mary's from 1948 to 1968, first as a PhD student and then on the staff, latterly as Reader in Biochemistry. His book 'The Biochemistry of Foreign Compounds' appeared in 1968, the year in which Parke took up the Chair of Biochemistry at the new University of Surrey, taking with him Jim Bridges and Lawrence King. They established a school, which made substantial contributions to the subject, notably developing an interface with toxicology. In London, Donald Davies moved to the Royal Postgraduate Medical School to join Colin Dollery's MRC Clinical Pharmacology Unit in 1967 upon his return from a postdoc with Brodie, and provided critical biochemical and analytical input into a series of groundbreaking studies, which impacted upon the development of clinical pharmacology in the U.K. and beyond. Davies' influence extended to Alastair Breckenridge, Charles George and Michael Rawlins. When they moved on to become heads of pharmacology in Liverpool, Southampton and Newcastle-upon-Tyne, respectively, they each developed departments in which drug metabolism and biochemical pharmacology were prominent. Upon his retirement in 1976, Williams was succeeded at St Mary's by Robert Smith, when the Medical School took the opportunity to move this now important and successful research from biochemistry into pharmacology by the creation of a Department of Biochemical and Experimental Pharmacology. By this time, drug metabolism was clearly seen as of great practical application to drug development and clinical use as well as underpinning the emerging science of mechanistic toxicology. It had thus left its historical base in biochemistry well behind. However, it has to be said that the move into pharmacology was for a time in the 1970s, in Britain at least, a contentious one. Despite this, the success of the move was secured in terms of the relevance of the field to medical teaching and a series of research achievements within a relatively short space of time.

Williams retired from the chair at St Mary's in 1976 and lived for only another three years, dying at the end of 1979, but awareness of his contributions remains. His biography is to be found in the Biographical Memoirs of Fellows of the Royal Society (Neuberger & Smith, 1982) and his Festschrift was published as 'Drug Metabolism from Microbe to Man' (Parke & Smith, 1977).

The Williams tradition continued at St Mary's through to 1998 under the leadership of Robert Smith and then the present author, when work in drug metabolism transferred to the South Kensington campus of Imperial College as part of the Faculty of Medicine formed by mergers with four London medical schools in 1997. Work in drug metabolism and toxicology is presently sustained at Imperial College with groups led by Jeremy Nicholson and Alan Boobis.

Eric Boyland, 1905–2002

In contrast with Williams' wide span of interests, almost the entire career of Boyland (Figure 3) focussed on the problem of chemical carcinogenesis. Another who came up the hard way, Boyland's early education was at night classes at the Manchester College of Technology while working as a technician at the British Alizarin Company, a dyestuffs company and one of the forerunners of the Pharmaceuticals Division of ICI (now AstraZeneca). A scholarship enabled him to complete his degree full time in 1926, swiftly followed by an MSc from the Manchester Medical School and his PhD in 1929 from the Lister Institute in London. After a year with Meyerhof at the Kaiser Wilhelm Institute in Heidelberg, he joined the Institute for Cancer Research in London, around the time that its director, Ernest Kennaway, identified

Figure 3 Eric Boyland in 1950.

benzol[a]pyrene as the principal carcinogenic component of soot.

Boyland was struck by the chemical inertness of these cancer-causing hydrocarbons, which gave rise to tumours at sites remote from their initial entry to the body. He postulated that they were converted by metabolism to more reactive compounds, which initiated the carcinogenic process.

This early proposal, made first in 1938, was followed by his 1950 suggestion that epoxides (arene oxides) were key intermediates in the metabolic hydroxylation of aromatic hydrocarbons. These ideas were at least 20 years ahead of their time but the synthesis of arene oxides in the 1960s led to these chemically reactive metabolites being established as the key to the metabolic activation of hydrocarbons, leading to DNA damage. The first confirmation of this appropriately came from Boyland's co-workers soon after his retirement in 1970: engaged in a race with at least two well resourced American laboratories, Peter Sims and Philip Grover established benzo[a]pyrene-7,8-diol-9,10-oxide as the proximate carcinogenic metabolite of Kennaway's prototype chemical carcinogen (Sims *et al*., 1974).

In addition to this almost prophetic work on epoxides, Boyland also contributed substantially to knowledge on the *N*-hydroxylation as a key step in the carcinogenicity of aromatic amines to the urinary bladder. This work was triggered by the very high incidence of bladder tumours in workers in the dyestuff and rubber industries exposed to aromatic amines. With Manson and Booth, Boyland established *N*-hydroxylation as one of many metabolic routes of carcinogens such as 2-naphthylamine and followed this by showing that *N*-hydroxylation was the key step in tumorigenesis. Studies of *N*-oxidation in the Boyland laboratory were later extended to tobacco alkaloids, a project that gave his then technical officer John Gorrod the springboard to an academic career. At the end of his active career from 1990 onwards, it was very fitting that Gorrod, by then Head of the Pharmacy Department at Chelsea College (now Kings College London) was able to offer an academic base close to Boyland's London home.

The mercapturic acid synthesis, the formation of *N*-acetylcysteine conjugates from halogenated aromatics, was discovered in 1879. Baumann & Preuss (1879), fed bromobenzene to dogs and discovered a sulphur-containing metabolite in the urine of the dogs, which upon hydrolysis yielded acetic acid and *p*-bromophenylmercaptan. They called this a 'mercapturic acid'. Independently, Jaffe (1879) found that chlorobenzene and iodobenzene also formed mercapturic acids. Baumann (1884) later reported the correct structure of the mercapturic acids as acetylcysteine conjugates. Over the years, a series of other classes of compounds were shown to undergo this conjugation, including sulphate esters, aromatic nitro compounds, etc.

However, the complete metabolic sequence leading to *N*-acetylcysteine conjugates remained unknown for over 70 years. The involvement of glutathione in the formation of mercapturic acids was established in 1959 by a team led by Sybil James and H.G. Bray in Birmingham. They first showed that administration of a mercapturic acid precursor led to a drop in liver glutathione levels commensurate with the amount of mercapturic acid formed (Barnes *et al*., 1959). They went on to show that *S*-(*p*-chlorobenzyl) glutathione was converted *in vitro* to *S*-*p*-chlorobenzylcysteine (Bray *et al*., 1959a) and that this compound could be acetylated by liver preparations (Bray *et al*., 1959b). After two years, Booth *et al*. (1961) described the direct enzymatic formation of glutathione conjugates in the cytosol of liver and other organs. This led to the work of Chasseaud and Boyland demonstrating the existence of distinct classes of glutathione transferases in the liver and other organs (see Boyland & Chasseaud, 1969). This provided in addition a pivotal role for Gowland Hopkins' tripeptide glutathione in xenobiotic metabolism and as a cellular defence mechanism against a wide range of toxins.

Upon his formal retirement from the Institute of Cancer Research in 1980, Boyland worked for two years at the International Agency for Cancer Research in Lyon and then took up a part-time but very committed post at the London School of Hygiene and Tropical Medicine, in the TUC Industrial Unit, which ended in 1990. His interest in science continued until his death at the age of 97 in 2002. A dedicatory volume of *Xenobiotica* appeared in 1986 (Volume 16 nos 10 & 11) which contained both autobiographical (Boyland, 1986) and biographical (Parke, 1986) material and a biographical memoir was published in 2000 (Boyland, 2000).

Geoffrey Dutton, b 1920

Glucuronic acid conjugation was discovered in 1879 and was rapidly established thereafter as an important pathway for endogenous and exogenous compounds alike. The substrates include bilirubin, steroids and other hormones as well as a plethora of drugs and toxic chemicals. Geoffrey Dutton (Figure 4) discovered the mechanism of glucuronide synthesis, specifically the role of the high energy intermediate uridine 5′-diphosphoglucuronic acid (UDPGA) as the source of glucuronic acid and the requirement for a microsomal UDP glucuronosyl transferase. This work, performed for his PhD under the supervision of Ian Storey, was the first unequivocal demonstration of the biochemical mechanism for any reaction of drug metabolism.

As a Scottish teenager, Geoffrey Dutton spent his Saturday afternoons in the arcane and chemically intriguing surroundings of a pre-World War II pharmacy and this may have influenced his decision to study biochemistry in Edinburgh immediately after the war. After graduating in 1948, Dutton

Figure 4 Geoffrey Dutton in 1982.

fell under the influence of G.F. Marrian, a steroid biochemist who had established the structures of pregnanediol and oestriol and shown that they were excreted as glucuronides. Marrian held the Chair of 'Chemistry in Relation to Medicine' in Edinburgh and his friendship with John Gaddum, then Professor of Pharmacology, provided one of the early stimuli to establish biochemical pharmacology in Britain. Marrian employed Dutton as a demonstrator in biochemistry, which allowed him to work part-time for a PhD under the supervision of Ian Storey on the enzymic formation of glucuronides. At that time, the ability of β-glucuronidase to cleave glucuronides was well established, but although some claimed it could also form the conjugates, this was contentious indeed and the mechanism of their formation was unknown.

Dutton and Storey started work in 1949, at a time when tissue homogenates were regarded with suspicion, in Europe at least. They benefited from a plethora of new techniques, which were revolutionising the life sciences, such as differential centrifugation and chromatography, but met with failure for a long time. They examined many possible sources for the energy to drive the conjugation without success. This changed on 21 February 1950 when they found a cofactor from slaughter-house liver, which supported the synthesis of 2-aminophenol glucuronide and followed this up with comparable findings for menthol glucuronide. When these findings were presented to the Biochemical Society in November 1950 (Dutton & Storey, 1951), R.T. Williams commented that they had found 'a big thing'.

But what was the cofactor? After laborious isolation procedures, chemical analysis showed the cofactor contained both stable and labile phosphorus, a pyrimidine nucleotide and a sugar. It resisted identification until they found an obscure paper showing that a uracil cofactor supported the conversion of glucose phosphate to galactose phosphate. This provided the clue that their cofactor was uridine-5-phosphate with pyrophosphate and an α-link to the glucuronic acid moiety, UDPGA. Their key paper (Dutton & Storey, 1954) provided the first molecular mechanism for a reaction of drug metabolism.

Rapidly following this discovery, the field suddenly contained many sugar nucleotides, but Dutton was not distracted from the glucuronidation theme. Upon moving to Dundee as a Lecturer in biochemistry (then part of physiology) under benign guidance of R.P. Cook, a product of Gowland Hopkins, Dutton took up the enzymic basis of glucuronide formation as the key topic of the remainder of his career.

Dutton and Storey's original findings were confirmed and extended in Dundee and elsewhere, a notable contributor at this early time being Julius Axelrod at NIH, winner of the Nobel Prize in 1970. This laid a firm foundation for remainder of Dutton's career and led to a School in Dundee whose influence continues to the present day.

The Dutton group was assiduous in their studies of the microsomal glucuronyl transferase, examining successively its substrate specificity, ontogenesis and control by various hormonal influences as well as differences in activity across species. Their careful classification of both endogenous and exogenous substrates and the study of the remarkable deficiency of the formation of certain glucuronides in the domestic cat presaged the multiplicity of the enzyme system, evidence strengthened by studies on the differential effects of enzyme inducers, as this phenomenon was exploited from the 1970s onwards. This was followed much later by protein heterogeneity studies and work on the differential regulation of the synthesis of various isozymes, which support the majority of their early conclusions.

Dutton edited two important volumes, 'Glucuronic Acid Free and Combined' (1966) and 'Glucuronidation of Drugs and Other Compounds' (1980), which remain landmarks in this field and are widely consulted to the present day. Over 50 years, the field that Dutton founded has moved from being a revolutionary novelty to one of the cornerstones of drug metabolism, as important as the cytochrome P450 system, which began in Britain and where British contributions are sustained today.

Dutton spent his entire academic career in the Biochemistry Department in Dundee where he produced a series of influential co-workers, including Ian Stevenson, Brian Burchell and Julian Leakey. As the subject developed, Brian Burchell led a move into the Medical School in Dundee in 1986, where he has been joined by Michael Coughtrie. Drug metabolism continues to flourish in Dundee, the impact of this group being added to by the recruitment in 1992 of Roland Wolf, who now leads the Biomedical Research Centre in the University of Dundee. Together, these contributions to drug metabolism play an important role in the overall excellence of Dundee in the biomedical sciences.

Dutton's festschrift appeared in *Transactions of the Biochemical Society* in 1984 and an autobiographical memoir was published in *Drug Metabolism Reviews* (Dutton, 1997).

Pharmacogenetics

The history of pharmacogenetics is shorter than that of drug metabolism since the term was only introduced in the 1950s. The first demonstration of marked individual differences in response to a drug was the association of malignant hyperthermia with general anaesthesia in the early 1950s by Kalow (see Kalow, 1970). *Pharmacogenetics* was originally defined as 'clinically important hereditary variation in response to drugs' by Vogel (1959) and the discipline was established by Kalow's monograph 'Pharmacogenetics' in 1962. Around this time, David Price Evans, then working in Baltimore, established the first genetic polymorphism of drug metabolism by a random family study and a small number of further examples accrued from the 1950s onwards, generally involving a small number of related individuals showing aberrant responses to a number of specific agents. The field underwent explosive growth from the mid-1970s with the discovery of what is now recognised as the genetic polymorphism of the microsomal monooxygenase CYP 2D6 emerging from work on the adrenergic neurone blocker debrisoquine by Robert Smith and co-workers in London and on the oxytocic alkaloid sparteine by Michel Eichelbaum in Bonn. This was the first genetic polymorphism whose frequency (about 7% among Caucasians) was such as to make it of relevance in the general population and also the first to affect a number of therapeutically significant drugs.

The British contribution to this field has been of critical importance, with early work providing its overarching framework and context and then 20 years apart two seminal sets of observations, which have helped place the field in its current prominence. Again, these contributions will be reviewed by examining the contributions of three key figures.

Figure 5 Sir Archibald Garrod about 1910.

Sir Archibald Garrod (1857–1936)

Archibald Garrod (Figure 5) is indisputably recognised as the father of genetic diseases. Garrod came from a family and educational background which provided the springboard from which he made the greatest individual contribution to the establishment of our modern discipline of medical genetics. His father, Alfred, was a distinguished Harley Street consultant, the foremost 19th Century investigator of rheumatic disease. With the aid of the first clinical laboratory test (for uric acid in plasma) he differentiated gout from the disease he named rheumatoid arthritis.

Although initially something of an underachiever, the younger Garrod emerged as a scholarly and disciplined man. His early interest in astronomy made him aware of the potential of spectroscopy, which he applied to the studies of urinary pigments leading to his elucidation of alkaptonuria.

Alkaptonuria is a rare familial disease of organic acid metabolism characterised by the darkening of urine to black after it is exposed to the air. In later life, affected individuals develop arthritis characterised by deposition of brown pigment in joint cartilage and connective tissue. Garrod studied the recurrence patterns in several families and realised it followed an autosomal recessive pattern of inheritance. He then postulated that it was caused by a mutation in a gene for an enzyme involved in the metabolism of a class of compounds called alkaptans, published in 1902 under the title 'The Incidence of Alkaptonuria: a Study in Chemical Individuality' (Garrod, 1902).

Over the next decade he extended his work to cover a number of other inherited diseases of metabolism, including cystinuria, pentosuria, and albinism. He formulated the 'one gene, one enzyme' hypothesis and described the nature of recessive inheritance of most enzyme defects. In 1908, the core of this work was presented as the Croonian lectures to the Royal College of Physicians, entitled *Inborn Errors of Metabolism*, subsequently published in book form (Garrod, 1909). At the time, these errors were viewed as 'metabolic sports' but it is now appreciated that, as well as being important in their own right, these inborn errors of metabolism provide a series of paradigms for a much wider range of disease aetiology.

Figure 6 David Price Evans in 1996.

Garrod was the first person to appreciate the biochemical individuality of humans. The rediscovery of Mendel around the turn of the 20th Century influenced his thinking, while he was clearly inspired by his close friendship with the doyen of biochemistry of his day, Frederick Gowland Hopkins (*vide supra*). The association of these two outstanding figures provides a model for the many fruitful relationships between clinical investigators and basic scientists which have followed.

At the end of his career, Garrod was appointed to the Regius Chair of Medicine at Oxford, upon the move of Sir William Osler to Johns Hopkins. The contrast between the two men was highlighted by McCarty (1994): Osler charming patients and students at the bedside, Garrod detached and chiefly interested in the patient's urine, perhaps the first scientifically orientated 'pisse prophet' of mediaeval medicine. Numerous accounts of his Garrod's career have appeared over the years, a modern biography of Garrod being published in 1993 (Bearn, 1993).

David Price Evans, b 1927

A Welsh-speaking Welshman, David Price Evans (Figure 6) read medicine in Liverpool, qualifying in 1951. During his undergraduate years, he acquired a strong scientific background, his interests in chemistry and biochemistry being stimulated by R.A. Morton in 1951, head of the Biochemistry Department: he obtained a 1st class BSc in 1948. After qualification in 1951, he spent a period with R.A. Gregory as a Holt Fellow in physiology, obtaining an MSc.

Price Evans had encountered Cyril Clarke as an undergraduate, but fell under his influence particularly after obtaining his MRCP in 1956. Clarke, later Sir Cyril Clarke FRS, was responsible for the emphasis on genetics in Liverpool medicine, aided by Philip Shepherd, Professor of Genetics and Richard McConnell, a physician with an interest in the impact of blood groups upon biochemical disease. Price Evans' first study under Clarke's patronage, which led to his PhD, was on L-fucose in saliva. This taught him a lot of practical bench chemistry and the importance of stringent statistical appraisal of results.

As a result of McConnell's acquaintance with Victor McKusick, Professor of Medicine at Johns Hopkins in Baltimore, Price Evans became the first of many young Liverpool clinicians to move between these two great Atlantic port cities, a connection that invigorated Liverpool medicine for a number of years.

McKusick introduced Price Evans to the problem of the wide intersubject variation in the rate of metabolism of isoniazid, at that time the leading drug for the treatment of tuberculosis. Hettie Hughes, in Cincinnati, had data suggesting that these were two types of people, 'fast' and 'slow' metabolisers. Victor McCusick realized that the two isoniazid phenotypes might be single gene Mendelian characters and that proving this would be a real achievement and also that Price Evans was ideally equipped to tackle the problem. First, his Liverpool background pointed to the need for chemical rather than microbiological assays of isoniazid. Second, his awareness that pedigree studies of random families would provide proof of Mendelian inheritance, deriving from his school days and reinforced by Clarke and Shepherd. Third, he had a deep understanding of the Hardy–Weinburg equilibrium, obtained from his exposure to the eminent statisticians Jerome Cornfeld and Curt Stein while at Johns Hopkins.

The critical study proved that the existence of a genetic polymorphism of drug metabolism could be shown using random families. Using the available crude estimates of allele frequencies and assuming that white American families would have 2.5 offspring each, it was easy to compute how many families would need to be tested. McKusick provided the clinical and laboratory resources for the study, which was successfully completed at the end of 1959 and was the basis of Price Evans' Liverpool MD (1960) as well as the Citation Classic 'Genetic control of isoniazid metabolism in man' (Evans *et al*., 1960), cited at least 538 times up to 4 July 2005. In his commentary on this paper, Price Evans stated that this was 'one of a small number of publications that formed the basis of an interdisciplinary branch of medicine termed 'Pharmacogenetics' … it is probable that this has been cited so often because it presented a clear-cut conclusion of interest to workers in different fields… human genetics, pharmacology, clinical medicine, toxicology and epidemiology' (Price Evans, 1987).

Price Evans joined the staff of the academic Department of Medicine in Liverpool soon after returning from Johns Hopkins. The large numbers of patients undergoing upper gastrointestinal surgery gave a source of human liver. These subjects were phenotyped with isoniazid preoperatively and their liver tissue was also phenotyped using sulphadimidine and the colorimetric Bratton-Marshall analysis. This gave definitive proof of the biochemical basis of the polymorphism as different forms of hepatic *N*-acetyltransferase, a theme taken up by others and used as the basis of demonstrations of both protein and genetic polymorphisms of this enzyme (see Weber, 2001). In recent years, the work of Edith Sim in Oxford has contributed particularly to the functional characterisation of novel polymorphisms associated with the genes for arylamine *N*-acetyltransferases.

Following these seminal studies, a series of other pharmacogenetic projects were pursued in Liverpool under Price Evans' direction, notably the discovery of the human paraoxonase polymorphism. He also collaborated with clinical pharmacologists at the Karolinska Institute on studies of variability in the metabolism of early tricyclic antidepressants, which provided strong suggestions of genetic polymorphisms in their metabolism. However, definitive proof of this was elusive.

Robert Smith and his co-workers at St Mary's were fully aware of the significance of Price Evans' work, through their interests in conjugation reactions and through Price Evans' friendship with his fellow Welsh speaker, R.T. Williams. When the first studies on debrisoquine suggested a possible new genetic polymorphism, it was to Price Evans that the St Mary's group turned for advice. He was a valued collaborator in the design and interpretation of the population and pedigree studies, which established the genetic polymorphism (Evans *et al*., 1980) and in confirming that the metabolism of sparteine and debrisoquine were governed by the same genetic polymorphism (Evans *et al*., 1983).

Price Evans left Liverpool in 1983, accepting the invitiation to be Director of medicine at the Riyadh Armed Forces Hospital in Saudi Arabia (a post he held for 16 years, then becoming Senior Physician). Although his opportunities for active research were reduced, he continues to follow actively the field he played a major part in founding. He was a member of the founding Editorial Board of the journal 'Pharmacogenetics' (now Pharmacogenetics and Genomics) in 1985 and in 1993 his *magnum opus* 'Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics 'was published by Cambridge University Press (Price Evans, 1993).

Robert Smith, b 1934

Robert Smith (Figure 7) left school at 16 to become a technician in the then-new Biochemistry Laboratory at Allen and Hanburys at Ware in Hertfordshire, where he was deeply impressed by seeing the life-saving purple band of Vitamin B12 moving down an alumina chromatography column. After this introduction to the facinating world of galenicals as well as proprietary medicines, he went to Chelsea School of Pharmacy where he was taught by some notable scientists including Mary Lockett and Arnold Beckett. After graduating in 1956, he served his pharmacy apprenticeship at Menley & James (later taken over by Smith Kline & French) in Coldharbour Lane,

Figure 7 Robert Smith in 1981.

Brixton where his apprentice master was David Jack (subsequently Sir David, research director of Glaxo and discoverer of salbutamol and ranitidine), prior to joining the Williams' Laboratory at St Mary's, obtaining his PhD in 1960. He then followed a well-trodden path to spend a period with Bernard Brodie in the Laboratory for Chemical Pharmacology at NIH before returning to be appointed as a lecturer at St Mary's in 1962. He was fortunate to be assigned to work on the drug thalidomide by Williams, which led to a very early exposure to the broader context of the contribution of metabolic studies to drug development and use. Smith's work through the 1960s and 1970s established him as one of the world's leading figures in the field, with seminal work on the biliary excretion of drugs, drugs of abuse and species differences in drug metabolism, as well as the discovery (with Margaret James) of a new pathway of metabolic conjugation, the taurine conjugation of arylacetic acids (James *et al*., 1972).

Smith has recorded his role in the discovery of the 'debrisoquine polymorphism' in his Paton Prize Lecture to the British Toxicology Society (Smith, 2001). As mentioned above, by the early 1970s there were a series of reports in the literature pointing to the possibility of genetic polymorphisms of drug hydroxylation but none were established.

At this time, the St Mary's laboratory had a well-established practice of self-experimentation, in which Smith had been an enthusiastic participant (if occasionally forgetful in collecting all the samples required). The late Graham Dring, then a lecturer in the department had declared that Smith was in some way 'metabolically odd', as he hydroxylated amphetamine much less extensively than Dring himself, and others in the laboratory (see Dring *et al*., 1970).

Towards the end of Tecwyn Williams' tenure, the interests of the St Mary's group began to include issues relating to human drug use. This led to discussions involving Dr Richard Lancaster, which focussed attention upon debrisoquine, an adrenergic neurone blocker that exhibited marked interpatient variation in antihypertensive response. Considerable interpatient variation in the extent of hydroxylation of debrisoquine was established and this low hydroxylation was associated with marked hypotensive activity. This led to a seminal experiment in May 1975 in which five volunteers took debrisoquine in a protocol linking serial blood pressure measurement and the assay of unchanged drug and 4-hydroxydebrisoquine in urine. Four subjects were relatively unaffected, but Smith became dizzy and faint and was unable to stand, as a result of severe orthostatic hypotension. His blood pressure fell as low as 70/50 mmHg and while this resolved within 4–5 h, cardiovascular effects persisted for 48 h. Analysis of urinary metabolites showed that Smith excreted debrisoquine almost entirely unchanged, in marked contrast to the other volunteers, who excreted principally 4-hydroxydebrisoquine.

This prompted the search for more nonmetabolisers and hyperresponders in the population. A total of 94 volunteers, largely students and staff at St Mary's, were examined (with a lower dose (10 mg) of debrisoquine to avoid the extreme effects seen in Smith) and this revealed a clear population polymorphism, with 8/94 being termed 'poor metabolisers' and the remainder 'extensive metabolisers', acronyms remaining in popular use now. Independently and concurrently with this work, parallel studies were being pursued with the oxytocic alkaloid sparteine, by Michel Eichelbaum, which revealed two subjects unable to metabolise the drug (Eichelbaum *et al*., 1975; 1979).

Eichelbaum and Smith presented their findings in the same session at the 6th International Congress of Pharmacology in Helsinki in 1975 and the back-to-back presentation of histograms showing the population distribution of the metabolism of the two drugs strongly suggested to many in the audience that they were examining two aspects of the same phenomenon. Unlike others who had studied drugs with multiple pathways of metabolism and elimination, like the tricyclic antidepressants (*vide supra*), Smith and Eichelbaum had fortuitously chosen to study drugs with a single dominant metabolic pathway and which thus provided excellent probes for a deficiency in the enzyme responsible.

These findings were confirmed and extended rapidly. Smith and co-workers recruited David Price Evans to assist in the design and interpretation of population and family studies to establish the fundamental genetic characteristics of the polymorphism. The results on the allelic frequency underlying the polymorphism drawn from their data have altered very little as the numbers of subjects studied have increased substantially in later years.

The existence of a genetic polymorphism affecting 7% of the population, which influenced the clinical response to affected drugs, provided a huge stimulus to drug metabolism on the interface with clinical pharmacology. The St Mary's group examined the impact of the polymorphism on the efficacy and adverse reactions of a series of important drugs. The work of Rashmi Shah on perhexiline is of particular note in this regard (Shah *et al*., 1982). The enzymic basis of the defect was established by others, facilitated by rapid progress from the 1970s on the multiplicity of the cytochrome P450 system, followed by molecular genetic studies that showed the multiplicity of variant alleles underlying the population polymorphism for P450 isozymes. With the emergence of a systematic nomenclature for the microsomal enzymes, the debrisoquine/sparteine polymorphism became known as the CYP2D6 polymorphism.

Following on from this work, Smith, who has always been fascinated by the more unusual aspects of drug metabolism, pursued the very rare metabolic disorder of trimethylaminuria or 'fish odour syndrome', in collaboration with Stephen Mitchell. The first case of trimethylaminuria was described in the medical literature in the 1970s, but literary references to patients suffering a strong fish-like body odour and halitosis go back a thousand years. This is often inaccurately diagnosed, with explanations ranging from poor hygiene to psychiatric problems and sufferers may withdraw from the outside world.

Trimethylamine (TMA) and its N-oxide (TMAO) are normal components of human urine, deriving from the diet and from the enterobacterial metabolism of precursors such as choline. Dietary TMA is almost entirely metabolised to and excreted as TMAO. Smith and Mitchell showed that the extent of TMA N-oxidation in a British white population study was polymorphic (Al-Waiz *et al*., 1987). Two propositi were identified with relative TMA N-oxidation deficiency. Family studies of the two propositi, as well as those of two identified subjects with trimethylaminuria, indicated that impaired N-oxidation is inherited as a recessive trait. In studies a decade later, in collaboration with Ian Phillips at Queen Mary College in London, Dolphin, Smith and others showed the molecular basis of the polymorphism (Dolphin *et al*., 1997). TMA oxidation is catalysed by the hepatic microsomal flavin-containing mono-oxygenase (FMO), and tissue localisation and functional studies have established FMO3 as the form

most likely to be defective in fish-odour syndrome. Sequencing FMO3 amplified from a patient with fish-odour syndrome identified two missense mutations, one a common polymorphism but the other, a C-T transition in exon 4, was found only in an affected pedigree, in which it segregated with the disorder. The latter mutation predicts a proline–leucine substitution at residue 153 and abolishes FMO3 catalytic activity.

Since the mid-1970s, further polymorphisms have been discovered, the list now including CYP2A6, 2C9 and 2C19 and extended to other enzyme systems, such as the glucuronyl and glutathione transferases and thiomethyl *S*-methyl transferase (see Evans, 2003). Many of these have clinical significance and the sequelae of the CYP 2D6 polymorphism as initially articulated by Idle & Smith (1979) continue to provide paradigms for the investigation and exploitation of these.

Robert Smith formally retired from his Chair at St Mary's in 1992 but continues to be active and influential in the field as a Senior Research Fellow at Imperial College. His 2001 Paton Lecture, referred to above, provides a partial account of his career.

Current status and future perspective

This deliberately selective short review has attempted to highlight the some of the remarkable contributions of some key British scientists to the development of drug metabolism and pharmacogenetics as we see them in 2006. As technology has developed, the nature of studies in drug metabolism has changed. With current analytical advances, characterising metabolites has long ceased to be the challenge it was when the author started work in the field in the 1960s. Similarly, the 'new biology' has revolutionised our ability to characterise the enzyme systems responsible and to study physiological and pathological factors that influence them. As a result, such work is nowadays largely in the province of the pharmaceutical industry and the contract research laboratories rather than academia and the numbers of university departments concentrating on drug metabolism and its implications have declined in the U.K. and across the world. However, the emphasis on human individuality in both disease susceptibility and response to treatment that has resulted from the Human Genome Project has placed drug metabolism once again at centre stage as an essential aspect of the development of 'personalised medicine'. The numerous examples of inherited differences in drug metabolising enzymes as well as other key systems such as drug transporters and receptors (outside the present coverage) emerge as principal determinants of both efficacy and toxicity. The long established principles of the field are being combined with molecular genetics to optimise drug therapy for individual patients. While this is at the earliest stage at present, there is a major intellectual challenge establish a new science which brings together diverse strands to develop the molecular diagnostics that will accompany existing and novel drugs to maximise benefit and obviate many of the adverse reactions that have plagued past generations of therapies. Paraphrasing Sir Isaac Newton, this review acknowledges some of the distinguished British scientists on whose shoulders those developing personalised medicine stand.

I am extremely grateful to those who have provided written and verbal recollections of the figures and events covered in this review. I am particularly appreciative of my correspondence with David Price Evans, who has been most generous in providing insights into his work in Baltimore and Liverpool now more than 40 years ago. It has been my privilege to have known and worked with all those mentioned (with the obvious exceptions of Gowland Hopkins and Garrod) and to have witnessed a number of the events mentioned, notably Smith's day of self-experimentation in May 1975 and the subsequent presentation by Eichelbaum and Smith of their two polymorphisms at the 1975 IUPHAR congress. In acknowledging all those who have helped and encouraged me in this work, I stress that errors and omissions are solely the responsibility of the author. I also thank Debra Nicholls for her help with the preparation of the manuscript.

References

AL-WAIZ, M., AYESH, R., MITCHELL, S.C., IDLE, J.R. & SMITH, R.L. (1987). A genetic polymorphism of the N-oxidation of trimethylamine in humans. *Clin. Pharmacol. Ther.,* **42,** 588–594.

BARNES, M.M., JAMES, S.P. & WOOD, P.B. (1959). The formation of mercapturic acids. 1. Formation of mercapturic acid and the levels of glutathione in tissues. *Biochem. J.,* **71,** 680–690.

BAUMANN, E. & PREUSS, C. (1879). Ueber bromphenylmercaptursaure. *Ber. deut. chem. Ges.,* **12,** 806–810.

BAUMANN, E. (1884). Ueber cystin und cystein. *Z. Physiol. Chem.,* **8,** 299–305.

BEARN, A.G. (1993). *Archibald Garrod and the Individuality of Man*. Oxford: Clarendon Press.

BOOTH, J., BOYLAND, E. & SIMS, P. (1961). *Biochem. J.,* **79,** 516.

BOYLAND, E. (1986). The metabolism of foreign compounds and the induction of cancer. *Xenobiotica,* **16,** 899–913.

BOYLAND, E. & CHASSEAUD, L.F. (1969). The role of glutathione and glutathione *S*-transferases in mercapturic acid biosynthesis. *Adv. Enzymol. Relat. Areas Mol. Biol.,* **32,** 173–219.

BOYLAND, M. (2000). *A Biochemist in Cancer Research. A Memoir of Eric Boyland*. Pasadena, CA: Backyard Press.

BRAY, H.G., FRANKLIN, T.J. & JAMES, S.P. (1959a). The formation of mercapturic acids. 2. The possible role of glutathionase. *Biochem. J.,* **71,** 690–696.

BRAY, H.G., FRANKLIN, T.J. & JAMES, S.P. (1959b). The formation of mercapturic acids. 3. *N*-Acetylation of *S*-substituted cysteines in the rabbit, rat and guinea pig. *Biochem. J.,* **73,** 465–473.

CONTI, A. & BICKEL, M.H. (1977). History of drug metabolism: discovery of the major pathways in the 19th century. *Drug Metab. Rev.,* **6,** 1–50.

DOLPHIN, C.T., JANMOHAMED, A., SMITH, R.L., SHEPHARD, E.A. & PHILLIPS, I.R. (1997). Missense mutation in flavin-containing mono-oxygenase 3 gene, FMO3, underlies fish-odour syndrome. *Nat. Genet.,* **17,** 491–494.

DRING, L.G., SMITH, R.L. & WILLIAMS, R.T. (1970). The metabolic fate of amphetamine in man and other species. *Biochem J.,* **116,** 425–435.

DUTTON, G.J. & STOREY, I.D.E. (1951). Glucuronide synthesis in liver homogenates. *Biochem. J.,* **48,** xxix.

DUTTON, G.J. & STOREY, I.D.E. (1954). Uridine compounds in glucuronic acid metabolism. I. The formation of glucuronides in liver suspensions. *Biochem. J.,* **57,** 275–283.

DUTTON, G.J. (1997). Raising the colors: personal reflections on the glucuronidation revolution 1950–1970. *Drug Metab. Rev.,* **29,** 997–1024.

EICHELBAUM, M., SPANNBRUCKER, N. & DENGLER, H.J. (1975). *N*-oxidation of sparteine in man and its interindividual differences. *Naunyn Schmiedebergs Arch Pharmacol.,* **287** (suppl), R94.

EICHELBAUM, M., SPANNBRUCKER, N., STEINCKE, B. & DENGLER, H.J. (1979). Defective *N*-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur. J. Clin. Pharmacol.,* **16,** 183–187.

EVANS, D.A., MANLEY, K.A. & MCKUSICK, V.A. (1960). Genetic control of isoniazid metabolism in man. *Br. Med. J.,* **2,** 485–491.

EVANS, D.A., HARMER, D., DOWNHAM, D.Y., WHIBLEY, E.J., IDLE, J.R., RITCHIE, J.C. & SMITH, R.L. (1983). The genetic control of sparteine and debrisoquine metabolism in man with new methods of analysing bimodal distributions. *J. Med. Genet.,* **20,** 321–329.

EVANS, D.A., MAHGOUB, A., SLOAN, T.P., IDLE, J.R. & SMITH, R.L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J. Med. Genet.,* **17,** 102–105.

EVANS, W.E. (2003). Pharmacogenomics: marshalling the human genome to individualise drug therapy. *Gut.,* **52** (suppl 2), 10–18.

FABRO, S., SCHUMACHER, H., SMITH, R.L., STAGG, R.B. & WILLIAMS, R.T. (1965). The metabolism of thalidomide: some biological effects of thalidomide and its metabolites. *Br. J. Pharmacol. Chemother.,* **25,** 352–362.

GARROD, A.E. (1902). The incidence of alkaptonuria: a study in chemical individuality. *Lancet,* **2,** 1616–1620.

GARROD, A.E. (1909). *Inborn Errors of Metabolism*. Oxford: Oxford University Press.

HOPKINS, F.G. (1913). The dynamic side of biochemistry. Presidential Address to the Physiological Section of the British Association for the Advancement of Science, Birmingham Meeting, 1913 (London 1913). *Rep. Br. Assoc.,* 62.

HOPKINS, F.G. (1921). An autoxidisable constituent of the cell. *Biochem. J.,* **15,** 286.

HOPKINS, F.G. (1923). An oxidative mechanism in the living cell. *Lancet,* **1,** 1251.

HOPKINS, F.G. (1929). Glutathione: a reinvestigation. *J. Biol. Chem.,* **84,** 269.

IDLE, J.R. & SMITH, R.L. (1979). Polymorphisms of oxidation at carbon centres and their clinical significance. *Drug Metab. Rev.,* **9,** 301–317.

JAFFE, M. (1879). Ueber die nach einfuhrung von brombenzol und chlorbenzol im organismus entstehenden schwefelhaltigen sauren. *Ber. Deut. Chem. Ges.,* **12,** 1092–1098.

JAMES, M.O., SMITH, R.L., WILLIAMS, R.T. & REIDENBERG, M. (1972). The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. *Proc. Roy. Soc. London B. Biol. Sci.,* **182,** 25–35.

KALOW, W. (1962). *Pharmacogenetics: Heredity and Response to Drugs*. Philadelphia: WB Saunders Co.

KALOW, W. (1970). Malignant hyperthermia. *Proc. Roy. Soc. Med.,* **63,** 178–180.

KAMMINGA, H. & WEATHERALL, M.W. (1996). The making of a biochemist I: Frederick Gowland Hopkins' construction of dynamic biochemistry. *Med Hist,* **40,** 269–292.

KELLER, W. (1842). Ueber Verwandlung der Benzoësäure in Hippursäure. *Ann.,* **43,** 108–111.

MITCHELL, S.C. & WARING, R.H. (1997). Drug metabolism at Birmingham England: the Thorpe years 1927–68. *Xenobiotica,* **27,** 127–146.

NEEDHAM, J. & BALDWIN, E. (eds) (1949). *Hopkins & Biochemistry*. Cambridge: Heffer.

NEUBERGER, A. & SMITH, R.L. (1982). Richard Tecwyn Williams 1909–1979. *Biogr Mem Fellows Roy Soc,* **28,** 685–717.

PARKE, D.V. & SMITH, R.L. eds (1977). *Drug Metabolism from Microbe to Man*. London: Taylor & Francis.

PARKE, D.V. (1968). *The Biochemistry of Foreign Compounds*. Oxford: Pergamon Press.

PARKE, D.V. (1986). Eric Boyland – a pioneer in cancer biochemistry. *Xenobiotica,* **16,** 887–898.

PRICE EVANS, D.A. (1987). This Weeks' Citation Classic. Current Contents November 23, 1987.

PRICE EVANS, D.A. (1993). *Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics*. Cambridge: Cambridge University Press.

SHAH, R.R., OATES, N.S., IDLE, J.R., SMITH, R.L. & LOCKHART, J.D. (1982). Impaired oxidation of debrisoquine in patients with perhexiline neuropathy. *Br. Med. J.,* **284,** 295–299.

SIMS, P., GROVER, P.L., SWAISLAND, A., PAL, K. & HEWER, A. (1974). Metabolic activation of benzo[*a*]pyrene proceeds by a diol-epoxide. *Nature,* **252,** 326–328.

SMITH, R.L. (2001). The discovery of the debrisoquine hydroxylation polymorphism: Scientific and clinical impact and consequences. *Toxicology,* **168,** 11–19.

VOGEL, F. (1959). Moderne probleme der Humangenetik. *Ergeb Inn Med Kinderheilkd,* **12,** 52–125.

WEATHERALL, M.W. & KAMMINGA, H. (1996). The making of a biochemist. II: The construction of Frederick Gowland Hopkins' reputation. *Med. Hist.,* **40,** 415–436.

WEBER, W.W. (2001). The legacy of pharmacogenetics and potential applications. *Mutat. Res.,* **479,** 1–18.

WILLIAMS, R.T. (1947). *Detoxication Mechanisms*. London: Chapman & Hall.

WILLIAMS, R.T. (1959). *Detoxication Mechanisms,* 2nd edn. London: Chapman & Hall.