ISSX CONFERENCE

The many facets of foreign compound metabolism

A review of the ISSX first European Symposium on Foreign Compound (Xenobiotics) Metabolism held at the University of Malta between February 24th and March 3rd, 1985.

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This review is divided into two parts; the first part of the review appearing in this issue of 'Tha Pharmacist' deals with the proceedings of the first two days of the symposium mainly dedicated to Professor Eric Boyland and his work together with contemporary methods in drug metabolism studies.

The second part of this review which deals with the final two days dedicated to the role of disposition studies in the safety evaluation of consumer products, including agrochemicals and to oral and poster free communications will appear in the next issue of this journal.

INTRODUCTION

The local academic and industrial communities were in a unique and privileged position towards the beginning of this year because the International Society for the Study of Xenobiotics (ISSX) in cooperation with the University of Malta organized its first European symposium on Foreign Compound Metabolism at the Assembly Hall, University of Malta, Tal-Qroqq.

It was therefore with great pleasure and interested anticipation that I accepted to attend and report on proceedings on behalf of the Chamber of Pharmacists.

Some of the 120 participants at the ISSX conference applaud one of the speakers at the main sessions. Prof. R.L. Smith is (2nd from right front row) and Dr. P. Johnson (4th from right) and Dr. J.W. Gorrod (6th from right).
Prof. A.H. Beckett is (1st from right second row).
Prof. D.V. Parke is (1st from left, fourth row).

(Courtesy of Allied Newspapers Ltd)

About 120 participants from 12 countries including the U.S. and Japan attended the 4-day conference during which 25 papers were presented at the main sessions whilst there were 11 oral free communications; 19 poster free communications were also presented. All these contributed to various interesting discussion sessions between the speakers and their audience.

The organising committee included P. Johnson, chairman (Smith, Kline & French Ltd., England), L. Chasseud (Huntington Research Centre, England), V. Ferrito (University of Malta), G. Gibson (University of Surrey, England), J. Gorrod (University of London, England), R.L. Smith, President of ISSX, ex-officio (St. Mary's Hospital Medical School, England).

The programme committee was under the chairmanship of J. Gorrod and included G. Brooks (University of Sussex, England), D. Howes (Unilever, England), A. Renwick (University of Southampton, England), W. Ritter (Bayer, West Germany) and V. Ferrito and G. Gibson.

ISSX PRESIDENT INTERVIEWED

During a most cordial interview, Professor R.L. Smith (Department of Experimental Pharmacology, St. Mary's Hospital Medical School, University of London, England), who incidentally is a pharmacist, now Biochemical Pharmacologist and the current President of ISSX, explained to your author the 'raison d'étre' of the Society. Indeed, ISSX is the 'umbrella' under which the ever-increasing research workers in the various disciplines involving the study of xenobiotics, be it, Biochemical Pharmacology, Toxicology, Cancer Research, the pharmaceutical, chemical, agricultural and food as also the perfume industries are to find a common ground for reference, discussion, possible problem solving, influencing changes in research trends, updating, and influencing regulatory bodies in formulating related legislation; indeed, conferencing.

The first ISSX symposium was held in Florida, California, USA in 1983; but, it was soon felt that a European conference would be more easily accessible to the many members interested in the study of xenobiotics which include drugs and also non-drug cmpounds.

GLOSSARY

Xenobiotic: or 'foreign compound'... not necessarily a compound "not normally present in the diet" or "to which the organism is not normally exposed"; indeed, the food we eat and the air we breathe contain a formidable array of compounds which today are all described as 'foreign'; the term 'Xenobiotic' attempts to overcome the 'problem'; a generic term such as 'foreign compound' or some equally indefinable term as 'xenobiotic' is needed to refer to those compounds which by custom are classed as 'foreign'.

Biochemical Pharmacology: the field wherein not only the molecular aspects of a drug, i.e., a chemical substance, usually foreign, are explained but also the changes which they cause in biochemical systems and cellular strategy; indeed, it is devoted to research into the development of biologically active substances and their mode of action at the biochemical and subcellular level.

Detoxification, Detoxication: while metabolism often results in the conversion of xenobiotics to less toxic polar products, some metabolites may be more toxic than the parent compound resulting in tissue damage and even the initiation of carcinogenesis. Thus the word 'detoxication' used to describe the metabolic reactions undergone by foreign compounds is capable of giving rise to misconceptions concerning the nature of the processes involved in the metabolism of foreign compounds. Problems associated with the use of the term are now generally recognised.

Metabonates: chemical breakdown products of metabolism distinguished from metabolites, products of enzymatic degradation.

Phase I metabolism: or pre-conjugation metabolism including oxidative, reductive and/or hydrolytic reactions.

Phase II metabolism: further 'detoxication' by conjugate formation and subsequent excretion.

PROFESSOR BOYLAND HONOURED

Amongst the many eminent personalities in their respective fields who were participating at the conference was Professor Eric Boyland, leading Biochemist, Toxicologist and Educator who was honoured by the University of Malta for his outstanding contribution to cancer research with the conferment of a degree of Doctor of Medicine and Surgery (Honoris Causa) at the start of the conference.

The conferment was made by The University Rector, Professor G.P. Xuereb; the Chairman of the Faculty Board of Medicine and Surgery, Professor E. Grech was the sponsor.

In an address during the conferment ceremony, Professor Victor Ferrito, Acting Head of the Department of Pharmacy, described the English Professor, who incidentally was celebrating his 80th birthday whilst in Malta, as a pioneer, founder and father of the study of xenobiotics. He said that Prof. Boyland has made several important observations, discoveries and postulations, later proved correct which had laid the foundations for the science of molecular toxicology.

Born in Manchester in 1905, Prof. Boyland graduated B.Sc., in 1926 and M.Sc. in 1928 from Manchester University.

Between 1928 and 1931 he was Grocers Co. Scholar and Beit Memorial Fellow at the Lister Institute for Preventive Medicine and at the Keiser Wilhelm Institute, where he worked on the biochemistry of muscle.

He was awarded the Ph.D. degree by London University in 1930 and was appointed Physiological Chemist to the Royal Cancer Hospital in 1931. In 1936, he was awarded the B.Sc. of the University of Manchester and was appointed Reader in Biochemistry. He was appointed Professor of Biochemistry of London University in 1945.

Prof. E. Boyland receiving the degree of Doctor of Medicine and Surgery (Honoris Causa) from Prof. G. Xuereb.
(Courtesy of Allied Newspapers Ltd).

Professor Boyland retired from his professorship at the Institute of Cancer Research in 1979 and became visiting professor in Toxicology at the School of Hygiene and Tropical Medicine at the University of London. He still holds this position.

Professor Ferrito said that Professor Boyland's suggestions, postulations and predictions on the **in vivo** behaviour of various types of xenobiotics were in advance of his time.

The 4-day conference started immediately after the conferment ceremony. The first day was devoted to topics related to Prof. Boyland's career in research and included the presentation of papers by several of his former students and research collaborators.

SCIENTIFIC APPROACH TO CANCER RESEARCH THROUGH CHEMISTRY

The first session chairman, Prof. D.V. Parke (University of Surrey, England) introduced the day's work by presenting an appreciation of Prof. Boyland's Life and Work, which can be summarily described as a rigorously scientific approach to cancer research through chemistry.

Appreciation of Prof. Boyland's Life and Work

Indeed, amongst other important breakthroughs, Boyland identified the link between nitrosamines and cancer, e.g. in the bladder and stomach; the relation between thiocyanate levels in the saliva of cigarette smokers and cancer during studies on tobacco alkaloids. In 1948, Boyland indicated the relation between alkylating agents and cancer; and on to glutathione conjugation, the cell's main defence against cytotoxic effects of electrophilic xenobiotics. The identification of glutathione-S-Aryl-transferase removed any doubt of the actual existence of this pathway in the metabolism of xenobiotics. Boyland **et al** (1957-58) also carried out useful work to unequivocally show the presence of mercapturic acids in the urine of experimental animals.

Thus in about fifty years of fruitful activity, Boyland has been ahead of his time in advocating today's accepted trend of Prevention of cancer by modification of social and personal customs, such as tobacco smoking and sunbathing and a decrease in the abuse and, or rather, the misuse of chemicals.

Boyland's elucidation of the metabolic pathways of polycyclic aromatic hydrocarbons without the benefits of modern characterization methods, such as High Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR), Mass Spectroscopy (MS), etc. considered as essential today to workers in this field was described as 'more an art than science' by Prof. P.L. Grover (Chester Beatty Laboratories, Institute of Cancer Research, London) during the presentation of his paper entitled Metabolism and Activation of Polycyclic Aromatic hydrocarbons.

Following the isolation of Benzpyrene from coaltar in 1933, Boyland (with Levy, 1935) studied the metabolism of anthracene and (with Wolf, 1950) of phenanthrene. The existence of simple epoxides as intermediary metabolites of polycyclic hydrocarbons and as intermediates in the formation of dihydrodiols, phenols and glutathione conjugates was originally proposed by him in 1950. Such epoxides were initially regarded as being involved in detoxication but evidence now shows that epoxides of various types are also the reaction species responsible for the carcinogenic and mutagenic effects of the parent hydrocarbons; the cytotoxicity of polycyclic hydrocarbons may also be due to these epoxides, which, although not yet identified, recent work has shown that benz-a-pyrene diol-epoxide can be metabolized to an even more reactive species that reacts covalently with microsomal protein or with thiols and that may be a triol-epoxide.

Metabolism of Aromatic Amines

Dr. J.W. Gorrod (Department of Pharmacy, Chelsea College, University of London) gave an overview of work carried out on the metabolism of aromic amines, which are widely used in the chemical, pharmaceutical, rubber and plastic industries and occur as natural sustances and/or their combustion products. Many aromatic amines are toxic — indeed, approximately 35% of neoplasms in workers in such industries as above are occupational — and hence, their metabolism has been extensively studied with a view to elucidating the mechanisms involved.

The oxidative metabolism of aromatic amines can occur at 3 sites on the molecule viz., the constituent nitrogen, the aromatic ring system, and the substituents on the amino group. These oxidative reactions are mediated by the cytochrome P-450 isozyme system although other enzymes may play an important role in organ directed toxicity. The parent amines and the primary metabolites can undergo conjugation with acetic, glucuronic and sulphuric acids or with glutathione. The conjugation reactions are reversible and in the case of acetylation this may influence the position of nuclear hydroxylation compared to the parent amine.

During the course of these metabolic processes reactive compounds are sometimes produced which may be involved initiating a toxic response in a biological system. Indeed, that N-hydroxylation of aniline leads to phenylhydroxylamine associated with methaemoglobinaemia was first shown by Kiese (1959). Cramer **et al** (1960) identified the carcinogen N-hydro-

xy-2-acetlyaminofluorene in rats after incubation of acetylaminofluorene. Japanese workers studying N-hydroxylation of heterocyclic systems have identified potent mutagens, now carcinogens, trace amounts of which may be formed after food pyrolises. Boyland and Levy have shown the relation between nitrosamines and bladder cancer.

It would be of interest to note at this point that Dr. Gorrod was the organiser of at least three symposia on the Biological Oxidation of Nitrogen in Organic Molecules at Chelsea College and had edited the proceedings of these international symposia. The Department of Pharmacy of the University of Malta had participated at the 2nd International Symposium on the Biological Oxidation of Nitrogen in Organic Molecules held at Chelsea College, University of London, U.K.,19-23 September 1977 and had presented a paper, published in the proceedings of the symposium, entitled 'The metabolism of pyrroles and Indoles: ring nitrogen oxidation' under the authorship of Prof. A. Jaccarini and Miss Mary Ann Felice B.Pharm., M.Phil.

Metablism of Tobacco Alkaloids

The metabolism of tobacco alkaloids was then discussed by Prof. Dr. A.H. Beckett, (Chelsea College, University of London) who has had longstanding connections with the Department of Pharmacy, University of Malta having examined many a B.Pharm. student (including the undersigned, also at M.Phil. level). Tobacco contains more than a dozen alkaloids containing the pyridine ring structure substituted in the 3-position with 5 and 6 cyclic ring systems containing the N-atom in the 2-position.

The important centres of primary metabolic attack are the pyridyl N-centre, and the second N-centre (aliphatic in most of the alkaloids) and its **alpha**-carbon-atom, which are then further metabolized or changed chemically to metabonates. The pyridyl nitrogen can be metabolically methylated but apparently not converted to the N-oxide unless the second N-centre is rendered virtually non-basic, i.e., as in cotinine. The aliphatic tertiary N-centre can be converted to the N-oxide metabolically e.g. in nicotine and N-methlyanabasine, whereas an aliphatic secondary N-centre can be oxidized metabolically to the corresponding hydroxylamine e.g., in anabasine, which is chemically changed to the metabonate nitrone.

Metabolite C-oxidation on the C-atom **alpha** to the aliphatic N-centre leads to chemically unstable alkanolamines and thus N-dealkylation or ring opening depending on which **alpha**-carbon atom is attacked. Metabolic oxidation of other than C-atoms **alpha** to the non-aromatic N-centre can also occur e.g., cotinine to hydroxycotinine. Stereochemical aspects are important; nicotine and many of the minor tobacco alkaloids contain an asymmetric centre.

Metabolism of Anticancer Drugs

The essential role played in determining both symptoms and antitumor selectivity by drug metabolism, for most clinically useful anticancer agents was pointed out by T.A. Connors (MAC Toxicology Unit, Medical Research Council Laboratories, Surrey, U.K.), during his presentation entitled 'Metabolism of Anti-Cancer drugs'. Examples of pro-drugs which are pharmacodynamically inert but which may be metabolized to active drug were given; their use to improve both pharmacokinetic properties and drug penetration in the tumour was also discussed. Other examples of anticancer agents where drug metabolism may lead to greater antitumour selectivity or conversely a poorer therapeutic index were made.

Indeed, a simple knowledge of drug metabolism is indispensable for positive cancer therapy although one must always bear in mind that the metabolism of anticancer agents may be responsible for unique toxic features.

Conjugation Reactions Involving Glutathione

The importance of the reducing and nucleophilic properties of glutathione (GSH) in the metabolism of xenobiotics was emphasized by Brian Ketterer (Courtuald Institute of Biochemistry, Middlesex Hospital Medical School, London). Indeed, 'Phase I' mixed function oxidations of xenobiotics produce both free radicals and electrophiles simultaneously.

As a reducing agent GSH plays an essential role by detoxifying oxidizing by-products of oxygen utilization through the agency of Se-dependant GSH peroxidase and certain GSH transferase isoenzymes. The success of GSH in the detoxification of electrophiles depends on: 1) the chemical properties of the electrophiles, e.g., their "softness" and "hardness"; 2) the extent to which their detoxification is catalysed by GSH transferase isoenzymes; and 3) the tissue under consideration since isoenzyme distribution differs from tissue to tissue.

Perspectives and Horizons

Rounding up the day's work, Prof. Boyland (TUC Centenary Institute of Occupation Health London School of Hygiene and Tropical Medicine, London) expressed the hope that means by which cancer could be prevented by modification or inhibition of metabolism of foreign compounds would be presented at the meetings; many carcinogenic compounds including polycyclic hydrocarbons, aromatic amines and nitrosamines are active only after 'metabolic activation'. The activating processes involve the misuse or incomplete use of 'detoxicating' reactions. Knowledge of the biochemical mechanisms by which these compounds are activated to cause initiation, promotion and progression was accumulated slowly over a number of years. Processes which involve induction of cancer are not removed by evolution.

IMPORTANCE OF CONTEMPORARY METHODS IN DRUG METABOLIC STUDIES

At the present time, the identification of metabolites excreted in the urine continues to play a major role in the study of foreign compound metabolism, but with an important difference, namely, that powerful new investigative procedures have become available during the last 30 years or so.

Extraction of Metabolites from Biological Fluids

The second day of the conference was dedicated to such contemporary methods; the first session, under the chairmanship of W. Ritter (Bayer, West Germany) was opened by M. Stewart (Royal Infirmary Drug Investigation Unit, Scotland), who discussed the extraction of metabolites from biological fluids.

It is generally felt amongst workers in this field, however, that the isolation and therefore, extraction of intermediates in metabolic studies still does present a problem; also, it is the extraction procedure which now contributes to the majority of imprecisions in a drug assay; yet, despite all this, relatively little effort has been put into methods for improving this stage of analysis. Although detection systems for drugs and toxins continue to improve rapidly in both sensitivity and specificity, radio or optical immunoassays now allowing the detection of drugs at levels of 10^{-12}M, for positive identification, a specific method of extraction is necessary.

Solid phase extractions have considerable theoretical advantages over solvent extractions which are likely to contribute impurities to the final extract even after careful distillation and purification with losses in sensitivity once the final identification step is reached. Automated solid phase systems now exist which allow rapid processing of batches with minor operator involvement, thus also curtailing costs and increased time requests. Indeed, the Dupont Prep centrifugal extraction system described has been found to provide a flexible answer to a wide variety of extraction problems such as elimination of streaking as in solvent extraction, selectivity, a must in cathecholamine studies and of course, the extraction of metabolites in toxicological **in vivo** studies.

Utilisation of Radiochemicals in Xenobiotic Metabolism

The utilization of radiochemicals in xenobiotic metabolism, especially the use of ^{35}S-labelled inorganic sulphate to study effectively the formation, identification and whole-body disposition of sulphoconjugates was very interestingly described by Prof. G. Powell (University College, Wales) the only lady amongst the speakers at this symposium.

The usefullness of the isolated perfused liver system receiving continuous infusions of inorganic [^{35}S] sulphate and the formation of sulphoconjugates under steady-state conditions were given particular attention. The liver plays a control role in the formation of sulphoconjugates of both xenobiotics and naturally-occurring compounds and has also a role in the partitioning of suphoconjugated metabolites between bile and blood. By utilising [^{14}C] Leucine, the effects of xenobiotics on normal intermediary metabolic pathways such as the synthesis of proteins, glycoproteins, lipids, and cholesterol may be investigated.

Electrochemical Methods in Xenobiotic Metabolite Analysis

Dr. H. Oeschlager (Institut für Pharmazeutische Chemie der Universitat Frankfurt, W. Germany) President of the Pharmaceutical Society of that country, showed how electrochemical methods, in particular, Differential Pulse Polarography and HPLC with ECD (Electron-Capture Detector) are approved for drug analysis **in vivo.** By functionalization reactions polarographically inactive molecules are accessible from electrochemical detection to a limit in the

range of 10ng/ml. Since pharmacokinetics influence the pharmacodynamic effect to a great extent, a successful therapy needs analytical data concerning plasma levels of an administered drug and its metabolites, as well as the concentration of these xenobiotics in the urine and faeces. One convincing advantage of polarography is the possibility to calculate the current signal if the reducation- or oxidation mechanism is known.

HPLC: Limitations and Possibilities

The need for analytical methods of increased sensitivity and stability in drug metabolism studies has coincided with the introduction of high pressure liquid chromatography (HPLC; also referred to as high performance or high speed liquid chromatography) as a routine method of separating and quantitating drugs and metabolites. K. Zech (Byk-Gulden, Pharmaceuticals, GFR) discussed the limitations and possibilities of this relatively "young" technique, an indispensable tool in drug metabolism studies particularly as: a) biofluids can be introduced directly onto the reversed phase column, without prior extraction, b) performance is at room temperature, permitting the analysis of thermally labile metabolites and c) it is non-destructive, permitting 100% recovery of all metabolites with optimized separation and detection systems.

Extensive use of different detection for sensitivity, as well as on line screening for drug metabolites by coupling HPLC with a diode array detector (DAD). Direct injections of large amounts of biofluids on an automated precolumn switching system may overcome such limitations as errors by loss of metabolites or formation of artefacts during routine analysis sample preparation.

Thin Layer Chromatography (TLC) is employed for various purposes in drug metabolism studies; for separation and purification of metabolites prior to their characterization by other analytical (spectral) methods; to help identify drugs of their metabolites if authentic reference samples are available for direct comparison of Rf values and in addition TLC has been used for quantitation of compounds which are not amendable to Gas Chromatographic analysis (GC) due to their instability or poor GC properties.

Detection and Quantification of Xenobiotics and Metabolites by HPTLC

During the session chaired by P. Johnson (Smith, Kline & French, England) W. Ritter proposed the use of High Performance Thin Layer Chromatography (HPTLC), an analytical procedure based on conventional TLC but considerably faster and much more reproducable and economical. High performance is obtained by combining the increased separation power of HPTLC plates coated with an optimized silica gel and the instrumental performance of sample application (up to 20 samples simultaneously) chromatographic development under controlled conditions and quantitation by scanning **in situ.** The speaker surveyed the advantages of HPTLC as a tool for separation, detection and quantitation of various types of xenobiotics, e.g., polycyclic aromatic hydrocarbons, preservatives, nitrosamines, antioxidants, insecticides, herbicides and phosphate pesticides, drugs and their metabolites with examples from recent literature and his own experimental studies in drug pharmacokinetics and metabolism.

Spectral Methods in Metabolite Structure Elucidation

T. Marten, (I.C.I. Pharmaceuticals, England) then reviewed the "array" of spectroscopies available to the researcher for use in metabolite structure elucidation. Indeed, although traditional methods for identification of metabolites are still used, they have evolved and though phase II metabolites consist of large, polar molecules, presenting special difficulties to the spectroscopist, developments in mass spectrometry, (MS), Nuclear Magnetic Resonance (NMR), Ultraviolet (UV), and Infra-Red (IR) spectroscopy have allowed many of these problems to be overcome, e.g., DAD's have made looking for metabolites in biological samples much easier and have provided structural information at the same time.

Judicious use of stable isotopes and corresponding spectroscopic technique has aided metabolite detection and identification particularly when looking at a specific problem such as the position of hydroxylation on an allycyclic ring NMR spectroscopy remains vital because it gives very specific information on structural and spatial arrangements of atoms and Mass Spectrometry remains one of the most important analytical innovations; electrical impact mass spectrometry (EIMS), chemical ionization mass spectrometry (CIMS) and — to a lesser extent, field desorption mass spectrometry (FDMS), are analytical techniques which are now routinely used in metabolism studies. If an electron impact or chemical ionisation mass spectrometer is coupled with a gas chromatograph and a data

system, the separation, identification and quantitation of minute amounts (nanograms or less) of drugs and their metabolites become relatively simple procedures.

Many examples of drug metabolism reactions involve stereoisomers (diastereoisomers or enantiomers) either as substrates or as products. The discrimination of isomeric subtrates is termed isomeric selectivity and the phenomenon is influenced by enzymatic factors as well as molecular properties. Since enantiomers differ only in their chiral properties (e.g., optical rotation, interaction with other chiral compounds) they are not resolvable by the usual chromatographic techniques.

Analytical Methods in the Study of Stereoisomers

Prof. Bernard Testa's (School of Pharmacy, University of Lausanne, Switzerland) lecture on 'Analytical Methods in the Study of Stereoisomers' focussed on developments in the chromatographic discrimination of enantiomers using either chiral reagents or chiral phases. Reaction of enantiomers with an optically pure derivatization reagent leads to the formation of diastereoisomers which are separable by TLC, GLC, but emphasis was made on HPLC and its applications in the analysis of enantiomeric substrates and metabolites. Chiral bonded HPLC phases have been used for such separations.

Use of Isolated Hepatocytes in Xenobiotic Metabolism Studies

W. Voelter (University of Tubingen, W. Germany), presented results after following the formation of metabolites from aminpyrine, 2-ethyl-3-(4-hydroxybenzyl) benzofuran, N-alkyl-substituted piperidiness, biphenyl and 3-(p-chlorophenyl)-1-phenyl pyrazole-4-acetic acid in different **in vitro** systems and **in vivo** experiments by HPLC. The metabolic pattern of these xenobiotics received upon incubation with a 10,000xg liver supernatent fraction, microsomes prepared by different methods and importantly hepatocytes, were compared with **in vivo** experiments of different species. The use of isolated hepatocytes was advocated, being relatively easy, and of low cost; it reduces animal experiments and circumvents trials in humans.

(To be continued)

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