Excursions in biomedical mass spectrometry

GRAHAM W. TAYLOR, LOUISE E. DONNELLY*, STEPHEN MURRAY & NIGEL B. RENDELL
Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, UK

- 1 Mass spectrometry (MS) has played a vital role in the research of the department of clinical pharmacology for over 25 years.
- 2 MS has been used for trace analysis of endogenous compounds and xenobiotics in plasma and urine, and also for a wide range of structural studies.
- 3 Examples of current applications are reported, including data from gas chromatography-mass spectrometry (GC-MS) assays for mevalonic acid, the identification of an antibiotic produced by *Pseudomonas aeruginosa* which is active against *Helicobacter pylori*, high pressure liquid chromatography (h.p.l.c)-electrospray MS studies on steroid sulphates, the aspergillus gliotoxin, gliotoxin, and ADP ribosyltransferase activity in human polymorphonuclear neutrophil leucocytes (PMNs). The value of electrospray MS in the molecular weight determination of proteins is exemplified by the analysis of human serum amyloid component P.

Keywords ADP-ribosylation *Aspergillus fumigatus* electrospray gliotoxin *Helicobacter pylori* mevalonic acid *Pseudomonas aeruginosa* SAP steroid sulphate

Introduction

There has been a mass spectrometry (MS) facility in the Department of Clinical Pharmacology for over 25 years [1, 2], where it has played an integral part in the department's research into the mechanisms of asthma, cardiovascular disease, drug action, mutagenicity and toxicity. This was one of the first clinical departments in the UK to recognize the value of the technique for clinical research, particularly in the analysis of endogenous mediators or drugs in biological fluids. The application of gas chromatography (GC)-MS for picogramme analyses of the inflammatory mediators such as the prostanoids was pioneered in this department. Examples include the confirmation that prostacyclin was not, as had been previously supposed, a circulating hormone in man [3], work on the differential inhibition of cyclooxygenase *in vivo* by aspirin [4], evidence for cyclooxygenase activation in chronic smokers [5], patients with acute asthma [6] and liver disease [7]. The contribution of specific forms of cytochrome P450 to drug elimination and the enzymic activation of drugs and chemicals to toxic or mutagenic metabolites *in vivo* and *in vitro* has been underpinned by highly specific and sensitive GC-MS assays. Using these assays, the

biochemical pharmacology group in the department were the first to demonstrate that livers from subjects classified as poor metabolizers of debrisoquine had a specific defect in one form of cytochrome P450, later identified as CYP2D6, which catalyses the 4-hydroxylation of the drug [8]. Other examples include work on the relationship between CYP2D6 polymorphism and lung cancer caused by cigarette smoking [9], and the role of the food-derived mutagenic amines, PhIP, MeIQx and DiMeIQx, in cancer [10]. The latter work will be outlined in the accompanying article by Gooderham and colleagues [11].

Mass spectrometry has also been heavily used for structure determination; much of this work has been carried out in collaboration with groups both on and off site. In the eicosanoid field, we have examined the synthesis and metabolism of eicosanoids by human fetal membrane preparations [12, 13] and characterised the lung-derived eosinophil chemotactic factor as a mixture of leukotriene B₄ and 8S,15S-dihydroxy-5,9,11,13 (Z,E,Z,E)-eicosatetraenoic acid [14]. Other structural studies have included identification of argiotoxin, a glutamate receptor antagonist present in the venom of orb web spiders [15], the dictyostelium differentiation inducing factor, DIF-1 [16] and its metabolites [17],

Correspondence: Dr G. W. Taylor, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN, UK.

* Present address: Department of Thoracic Medicine, National Heart and Lung Institute, Royal Brompton Hospital, Dovehouse Street, London SW3, UK.

the demonstration that the *O*-glycans of *Toxocara* excretory-secretory antigen are *O*-methylated [18], and identification of metabolites of the carcinogen, 3-hydroxychrysene [19]. As part of a very fruitful collaboration with the Host Defence Unit at National Heart and Lung Institute, London we have identified a number of toxins released by *Pseudomonas aeruginosa* (including the ciliotoxin, pyocyanin, and the mucus secretagogues, the rhamnolipids) which contribute to the pathogenesis of the organism in human lung disease. These toxins are present in the airways of colonised patients (e.g. those with cystic fibrosis) at levels high enough to evoke biological responses [20, 21].

In 1994, we were successful in obtaining funds from the Wellcome Trust to update the MS facility, and installed two new mass spectrometers: a VG Quattro II triple quadrupole MS and a VG Trio 1000. Some examples of recent and current applications of mass spectrometry are outlined below.

Gas chromatography-mass spectrometry: mevalonic acid and cholesterol biosynthesis

Hypercholesterolaemia is a well recognised risk factor for coronary disease. Mevalonic acid (MVA) is an precursor of cholesterol, and it is now accepted that

fasting plasma MVA is a good indicator of cholesterol synthesis *in vivo*. In collaboration with Drs R. Naoumova and G. Thompson (MRC Lipoprotein Team, Hammersmith Hospital) we have developed a GC-MS assay to measure plasma MVA [22]. The assay is based on high resolution capillary GC coupled to electron capture (EC) MS. Plasma is spiked with the internal standard ($^2\text{H}_3$ -MVA) and extracted (using both solvent and solid phase methods). Derivatisation to the di-*O*-trimethylsilyl-*bis*-(trifluoromethyl)benzyl ester is undertaken followed by analysis by GC-ECMS in the selected ion monitoring mode. Ions are monitored at m/z 291 for MVA ($\text{M}-[(\text{CF}_3)_2\text{C}_6\text{H}_3\text{CH}_2]^-$) and m/z 294 for the internal standard. Using the technique, the fasting basal level of MVA in normolipidaemic subjects (at 09.00 h) was measured at $6.1 \pm 2.1 \text{ ng ml}^{-1}$ and exhibited the expected diurnal variation (Figure 1). MVA is formed by the action of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. Inhibitors of this enzyme (such as simvastatin) reduce endogenous cholesterol synthesis and increase low density lipoprotein (LDL) receptor activity leading to a fall in LDL cholesterol. Although the statins are used routinely in the clinic to treat patients with primary hypercholesterolaemia, there appears to be an individual variability in the LDL lowering response to the drugs. We have measured plasma MVA in patients with familial hypercholesterolaemia (FH). Basal MVA levels are within the normal

Figure 1 Mevalonic acid, the precursor of cholesterol, is assayed as the di-*O*-trimethylsilyl-*bis*-(trifluoromethyl)benzyl ester by gas chromatography-electron capture mass spectrometry. Ions are monitored at m/z 291 for MVA ($\text{M}-[(\text{CF}_3)_2\text{C}_6\text{H}_3\text{CH}_2]^-$) and m/z 294 for the internal standard. The diurnal variation in plasma MVA is also shown.

range and fall following treatment with a number of the HMG-CoA reductase inhibitors (simvastatin, pravastatin and atorvastatin). Patients who responded poorly to statins (with a relatively small decrease in LDL cholesterol) had a lower basal level of plasma MVA than those whose LDL cholesterol was more markedly reduced. These data suggest that the greater the basal rate of cholesterol synthesis in FH patients, the greater the LDL lowering response to statins [23]. Plasma MVA was raised in patients with non-insulin dependent diabetes mellitus (NIDDM) ($\sim 10 \text{ ng ml}^{-1}$). MVA fell following 13 h of hyperinsulinaemic, euglycaemic clamp but the fall was less marked in these patients compared with normal controls. This suggests that acute hyperinsulinaemia in the presence of euglycaemia decreases cholesterol synthesis less in NIDDM patients than in normal subjects [24]. It is known that high concentrations of insulin *in vivo* can stimulate LDL uptake with a consequent inhibition of HMG-CoA reductase activity [25], and the enhanced cholesterol synthesis in these patients may be due, in part, to a loss in sensitivity to the inhibitory effect of insulin.

Structure elucidation: A novel antihelicobacter agent from *Pseudomonas aeruginosa*

Peptic ulcer disease and gastritis are associated with colonisation by the microaerophile *Helicobacter pylori*. It has been widely debated whether the organism is causative of gastritis or merely a commensal that colonises inflamed mucosa: several findings suggest that it is causal, in particular, when *H. pylori* is eradicated in patients with gastritis or ulcers, there is a significant fall in recurrence rates [26]. The currently available therapy is only partially effective in eradicating this bacterium. It was noted that colonisation by *H. pylori* in patients with pernicious anaemia decreased as the disease progressed to end stage atrophic gastritis; this was associated with colonisation of the gastric mucosa with commensal flora from the mouth [27]: species present included staphylococci, klebsiella spp., streptococci spp. and pseudomonas spp. It was an intriguing possibility that the production of extracellular substances by colonising bacteria lead directly to the eradication of *H. pylori*. This led to the collaborative study with Dr S. Lacey (Department of Infectious Disease, Royal Postgraduate Medical School) into identification of a novel antihelicobacter substance. Ten clinical isolates of *P. aeruginosa* were tested against five strains (both metronidazole sensitive and resistant) of *H. pylori* using the cross streak assay. Although only 5/10 strains of *P. aeruginosa* were active in the assay, these inhibited all test strains of *H. pylori*. The antihelicobacter activity present in culture filtrates of one strain (PYO5) was completely extracted into chloroform and the extract was purified further by reverse phase high pressure liquid chromatography (h.p.l.c.). Antihelicobacter activity (as determined in a well plate assay) was associated with two partially resolved A_{254} u.v.-absorbing substances (fractions Ia, Ib), which were well separated from pyocyanin and 1-hydroxyphenazine

(Figure 2). The u.v. absorption spectra of fractions Ia and Ib were similar, both exhibiting a λ_{max} at 313 and 327 nm. The fast atom bombardment mass spectrum of #Ia gave intense $M+H^+$ ions at m/z 272 with lower intensity ion at m/z 270; these ions correspond with molecular masses 269 and 271. In fraction Ib, the ratio of m/z 272/270 was markedly reduced, implying that the second component of fraction I (Ib) was the dehydro form of Ia. Fraction #Ia eluted as a single peak on GC and generated an electron impact (EI) mass spectrum with intense ions at with ions at m/z 271 (M^+) 256 ($-\text{CH}_3$), 242, ($-\text{C}_2\text{H}_5$), 228 ($-\text{C}_3\text{H}_7$), 214 ($-\text{C}_4\text{H}_9$), 200 ($-\text{C}_5\text{H}_{11}$), 186 ($-\text{C}_6\text{H}_{13}$), 172 ($-\text{C}_7\text{H}_{15}$), 159 ($-\text{C}_8\text{H}_{16}$, base peak) and 130. These data are consistent with known pseudomonas products, 4-hydroxy-2-nonylquinoline and its nonenyl analogue [28]. Using the commercially available heptyl homologue, 4-hydroxy-2-heptylquinoline, less than $0.1 \mu\text{g}$ was required to cause significant inhibition in the well plate assay. The agar plate incorporation minimum inhibitory concentration (MIC) was determined for both metronidazole sensitive and resistant strains of *H. pylori* as $\sim 0.1 \mu\text{g ml}^{-1}$. This compares favourably with the reported value for metronidazole on sensitive strains at $1\text{--}2 \mu\text{g ml}^{-1}$. This material is currently being tested for efficacy against *H. pylori* in an animal model *in vivo*. *P. aeruginosa* also generates a number of other 4-hydroxy alkyl/alkenyl quinolines (and their respective *N*-oxides) with alkyl chain lengths varying from C_1 to C_{13} [29]. The relative potency of these homologues against *H. pylori* has not been determined. The mode of action of this antibiotic against *H. pylori* is not clear. The efficacy of the quinolines against both metronidazole resistant and sensitive strains of *H. pylori* may give an indication as to its site of action. It appears that metronidazole sensitivity is associated with anaerobic reductive activation of the drug, but this does not occur in resistant strains. Any effect of the hydroxyalkylquinolines must therefore occur through an unrelated mechanism. As there is no evidence that the quinolines can undergo facile substrate cycling (with the subsequent generation of O_2^- and $\text{OH}^\cdot$), the most likely locus for inhibition is in the electron transport chain, as it has been reported that these quinolines can inhibit electron transport at the cytochrome bc_1 complex in a number of respiring bacteria.

H.p.l.c.-MS

GC-MS has been the method of choice for the quantitation and identification of lipophilic species. Although derivatisation will impart volatility and thermal stability to many compounds including the prostanoids, mevalonate and mutagenic amines, some compounds, in particular many drugs and their metabolites are intractable to direct GC-MS analysis. Direct analysis of these polar materials by h.p.l.c.-mass spectrometry (h.p.l.c.-MS) can however be used. Until recently, the most successful approach to coupling h.p.l.c. and MS has been *via* the thermospray interface [30]. This interface requires heating of the vaporiser

Figure 2 *Pseudomonas aeruginosa* generates factors which inhibit the growth of *Helicobacter pylori* *in vitro*. Antibiotic activity elutes as a single peak on h.p.l.c. Mass spectrometric examination by fast atom bombardment, electron impact and (as the bis-(trifluoromethyl)benzoyl derivative) electron capture modes allowed the identification of the antibiotic as 4-hydroxy-2-nonylquinoline and its nonenyl analogue. Other 4-hydroxy-2-alkylquinolines (and their N-oxides) which are generated by *P. aeruginosa* elute separately on h.p.l.c.

probe, which can cause decomposition of thermally labile compounds, and problems with ion current stability are often encountered. The VG Quattro II is fitted with the recently developed electrospray interface which readily accepts aqueous solvents at flows of up to $200 \mu\text{L min}^{-1}$: this makes it ideal as an h.p.l.c. interface for the analysis of polar, thermally labile species, particularly if the added benefits of increased sensitivity of microbore h.p.l.c. columns are included. Examples of three recent studies using h.p.l.c.-electrospray MS are given below.

Steroid sulphates

With the increasing abuse of steroids in both athletic and equine sports, there is a growing need for more sensitive and specific methods of detection of these compounds. Steroid sulphates are products of steroid metabolism and may be used as markers of steroid administration as well as endogenous steroid production. Prior hydrolysis of a sulphate conjugate to the parent steroid before analysis, and this has a number of drawbacks, not least of which are problems of incomplete hydrolysis and degeneration of the liberated steroid during the hydrolytic procedure. Direct analysis of

steroid conjugates by h.p.l.c.-MS overcomes these problems as is exemplified by the analysis of seven steroid monosulphates. Five of the components in the mixture were fully resolved by h.p.l.c. and detected in the relevant ion channel with a signal to noise ratio of $>10:1$: oestrinol 3-sulphate (m/z 367, 2.13 min), cortisol 21-sulphate, (m/z 441, 4.40 min), 17β -oestradiol 17-sulphate (m/z 351, 5.89 min), dehydroisoandrosterone sulphate (m/z 367, 13.17 min) and 17α -hydroxy-5 α -androstan-3-one 17-sulphate (m/z 369, 16.30 min). The two unresolved compounds, testosterone 17-sulphate (m/z 367, 8.60 min) and oestrone 3-sulphate, m/z 349, 8.74 min) could be readily differentiated by differences in their mass spectra. The ion chromatograms were obtained on 100 pg of each analyte in the mixture injected on column which was sufficient to obtain full mass spectra post h.p.l.c. [31].

Aspergillus gliotoxin

Aspergillus fumigatus is a widely distributed saprophytic fungus. It can colonise the lungs of patients with compromised pulmonary host defences [32]. Compromising the mucociliary escalator is one common mechanism by which air borne pathogens can evade

Figure 3 *Aspergillus fumigatus* generates factors which slow human nasal ciliary beat frequency (CBF) *in vitro* and lead finally to epithelial disruption. Low molecular weight activity was identified by h.p.l.c.-electrospray MS as gliotoxin ($M+H^+$: m/z 327 together with a fragment at m/z 263). The inactive metabolite, bis-dethio-bis-(methylthio)gliotoxin ($M+H^+$: m/z 357 plus m/z 309) eluted immediately before gliotoxin, but could readily be identified by its characteristic mass spectrum.

clearance in the lungs (and this is one of the primary interests of Dr R. Wilson and Professor P. Cole at the Host Defence Unit, National Heart and Lung Institute, London). *A. fumigatus* generates numerous secondary metabolites, one of which, gliotoxin, is a potent ciliotoxic agent, causing a reduction in ciliary beat frequency (CBF) and leading eventually to epithelial disruption. It is metabolised by reductive methylation to bis-dethio-bis-(thiomethyl)-gliotoxin which has no biological effect on CBF. Neither gliotoxin nor its metabolite are amenable to GC-MS analysis, however, they were readily identified in culture filtrates of *A. fumigatus* by microbore h.p.l.c.-electrospray MS (Figure 3) [33]. We are currently examining sample of bronchoalveolar lavage and sputum from colonised patients in order to define the role of gliotoxin in aspergillus related lung disease in man.

ADP ribosylation

H.p.l.c.-electrospray MS has also been used to identify arginine-specific mono(ADP-ribosyl)transferase activity on the surface of human polymorphonuclear neutrophil leucocytes (PMNs) in collaboration with Professor J. MacDermot (Clinical Pharmacology, RPMS). The

pseudo-substrate, diethylamino(benzylideneamino)guanidine (DEA-BAG) was used as a ADP-ribose acceptor. The biological aspects of this work, together with a more extensive review of ADP ribosylation in signalling pathways, has been outlined in an accompanying paper by Allport and colleagues [34]. Following incubation of DEA-BAG with intact PMNs (and an initial h.p.l.c. purification), two ADP-ribosyl DEA-BAG anomers were observed in the h.p.l.c.-MS profile (Figure 4); analysis of the electrospray mass spectrum of peaks I and II indicated that the major ion species was m/z 775 corresponding to the protonated molecular ion ($M+H^+$) of the anomers; these peaks were absent in the control incubations with no cells. Further control incubations showed that ADP-ribosylation was not due to the reaction between DEA-BAG and free ADP-ribose (generated by hydrolysis of NAD^+ by an NAD^+ glycohydrolase). These data showed that human PMNs contain an arginine specific ADP ribosyl transferase on their surface [35].

Protein analysis

The mass range of the Quattro II is 4000 u, and thus it would appear, at first sight, to be of little value for the

Figure 4 Arginine specific ADP ribosyl transferase activity on the surface of human polymorphonuclear neutrophil leucocytes convert the pseudo substrate, diethylamino(benzylideneamino)guanidine (DEA-BAG) to ADP-riboseyl DEA-BAG (a). Two anomers I, II were observed in the h.p.l.c.-electrospray MS profile (c); analysis of the electrospray mass spectrum of peaks I and II indicated that the major ion species was m/z 775 corresponding to the protonated molecular ion ($M + H^+$) of the anomers (d). These peaks were absent in the control incubations with no cells (b). Further control incubations showed that ADP-riboseylation was not due to the reaction between DEA-BAG and free ADP-ribose.

molecular weight determination of biopolymers. Electrospray ionisation has overcome this limitation, and has made it possible to obtain molecular weight data on proteins of M_r 10 000–150 000. The sample, dissolved in a suitable solvent such as acetonitrile:water is injected directly into the ion source whereupon ionisation occurs to form multiply charged ions (with $z=5-50$); following mass analysis, these appear as a series of ions with m/z ratios well within the mass range of the instrument. Deconvolution, by a computer algorithm, converts the ion series back into a reconstructed molecular weight. The technique is exemplified by the spectrum of human serum amyloid P component (SAP) [36, 37] carried out by Dr W. Hutchinson (Immunological Medicine, RPMS). SAP with a molecular weight (M) of 25 462 protonates to generate the series of ions $(M + 16H)^{16+}$, $(M + 17H)^{17+}$, $(M + 18H)^{18+}$... $(M + 23H)^{23+}$, $(M + 24H)^{24+}$ (labelled $A_{16}-A_{24}$ with 16–24 charges respectively) appearing at m/z values of 1592–1062 (Figure 5a); the relationship between these charged states allows a computer extrapolation to determine the molecular weight of SAP as $25\,462.2 \pm 1.1$ (Figure 5b) which is in excellent agreement with the calculated molecular weight determined from the known sequence. The technique, which can generate good quality data on <20 pmol of protein in under 10 min is ideally suited for the examination of protein

variants, where single amino acid changes can readily be detected by the alteration in mass. Using electrospray MS structural differences in variants of amyloid proteins such as amyloid component P, apolipoprotein A_1 and lysozyme are now routinely determined in Professor Pepys's group in Immunological Medicine. Electrospray MS is also suited for molecular weight determination of peptides, the examination of protein digests. As can be seen from section 3 above, direct h.p.l.c.-MS is also proving invaluable in structural studies, and with Dr H. Stauss and Professor R. Lechler in the Department of Immunology, we are currently examining h.p.l.c.-electrospray MS, making use of the tandem MS facility for the characterisation of mixtures of MHC class II peptides.

Then and now: mass analysis from 120 u to 25 000 u

In 1970, mass spectrometric studies in the department were undertaken on an AEI MS12 magnetic instrument using packed GC columns. Only the 'classical' modes of ionisation—electron impact and positive ion chemical ionisation—were available, and research focussed on the analysis of drugs and their metabolites. Over the ensuing two and a half decades, under the stewardship of Harry Draffan, Tom Baillie, Ian Blair and currently, the

Figure 5 Electrospray mass spectrum of human serum amyloid P component. SAP generates a series of multiply charged ions (A_{16} – A_{24} with 16–24 charges respectively) appearing at m/z values of 1592–1062 (a). These data are transformed to give the molecular weight of SAP as $25\,462.2 \pm 1.1$ (b).

authors, the instrumentation has improved (and certainly become more user friendly) and the direction of MS research has adapted to the changing needs of the Department and the School. Electrospray ionisation and the ability to couple an h.p.l.c. directly to the mass spectrometer has revolutionised the application of biomedical MS. In 1970, Dollery and colleagues [1] were analysing low molecular weight, volatile fluorocarbons ($M_r=120$)—today we can determine protein molecular weights of $M_r > 25\,000$. We look forward to a similar increase in our operational ability over the next 25 years.

We are grateful to the Wellcome Trust for their generous funding of the VG Quattro II and Trio 1000 mass spectrometers. Some of the early GC-MS studies were undertaken on a Finnigan 4500 provided by the Medical Research Council. The work outlined above has arisen through long standing collaborations with a number of groups. We would like to thank our collaborators, particularly Professor J. MacDermot (Clinical Pharmacology, RPMS), Dr W. Hutchinson and Professor M. B. Pepys (Immunological Medicine, RPMS), Dr S. Lacey (Infectious Diseases, RPMS), Dr H. Stauss and Professor R. Lechler (Immunology), Dr R. Naumova and Dr G. Thompson (MRC Lipoprotein Group, Hammersmith Hospital), and Professor P. Cole, Dr A. Ryochi and Dr R. Wilson from the Host Defence Unit, National Heart and Lung Institute, London.

References

- 1 Dollery CT, Davies DS, Draffan GH, Williams FM, Conolly ME. Blood concentrations in man of fluorinated hydrocarbons after inhalation of pressurised aerosols. *Lancet* 1970; **ii**: 1164–1166.
- 2 Chalmers JP, Davies DS, Draffan GH, Reid JL, Thorgeirsson SS. Demethylation of 3-*O*-methyldopa. *Br J Pharmacol* 1971; **43**: 455P–456P.
- 3 Blair IA, Barrow SE, Waddell KA, Lewis PJ, Dollery CT. Prostacyclin is not a circulating hormone in man. *Prostaglandins* 1982; **23**: 579–589.
- 4 Heavey DJ, Barrow SE, Hickling NE, Ritter JM. Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature* 1985; **318**: 186–188.
- 5 Barrow SE, Ward PS, Sleightholm MA, Ritter JM, Dollery CT. Cigarette smoking: profiles of thromboxane- and prostacyclin-derived products in human urine. *Biochim Biophys Acta* 1989; **993**: 121–127.
- 6 Taylor IK, Ward PS, O'Shaughnessy KM, *et al.* Thromboxane A_2 biosynthesis in acute asthma and after antigen challenge. *Am Rev Respir Dis* 1991; **143**: 119–125.
- 7 Moore K, Ward PS, Taylor GW, Williams R. Systemic and renal production of thromboxane A_2 and prostacyclin in decompensated liver disease and hepatorenal syndrome. *Gastroenterology* 1991; **100**: 1069–1077.
- 8 Davies DS, Kahn GC, Murray S, Brodie MJ, Boobis AR. Evidence for an enzymatic defect in the 4-hydroxylation of debrisoquine by human liver. *Br J Clin Pharmacol* 1981; **11**: 89–91.
- 9 Speirs CJ, Murray S, Davies DS, Biola Mabadeje AF, Boobis AR. Debrisoquine oxidation phenotype and susceptibility to lung cancer. *Br J Clin Pharmacol* 1990; **29**: 101–109.
- 10 Lynch AM, Knize MG, Boobis AR, Gooderham NJ, Davies DS, Murray S. Intra- and interindividual variability in systemic exposure in humans to 2-amino-3, 8-dimethylimidazo[4,5-f]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, carcinogens present in cooked beef [see comments]. *Cancer Res* 1992; **52**: 6216–6223.
- 11 Gooderham NJ, Murray S, Lynch AM, *et al.* Heterocyclic amines: evaluation of their role in diet associated cancer. *Br J Clin Pharmacol* 1996; **42**: 91–98.
- 12 Sullivan MH, Roseblade CK, Rendell NB, Taylor GW, Elder MG. Metabolism of prostaglandins E2 and F2 alpha by human fetal membranes. *Biochim Biophys Acta* 1992; **1123**: 342–346.
- 13 Zosmer A, Rendell NB, Taylor GW, Elder MG, Sullivan MHF. Formation and metabolism of 14,15-epoxyeicosatrienoic acid by human reproductive tissues. *Biochim Biophys Acta* 1995; **1258**: 234–240.
- 14 Sehmi R, Cromwell O, Taylor GW, Kay AB. Identification of guinea pig eosinophil chemotactic factor of anaphylaxis as leukotriene B4 and 8(S),15(S)-dihydroxy-5,9,11, 13(Z,E,Z,E)-eicosatetraenoic acid. *J Immunol* 1991; **147**: 2276–2283.
- 15 Budd T, Clinton P, Dell A, *et al.* Isolation and characterisation of glutamate receptor antagonists from venoms of orb-weaver spiders. *Brain Res* 1988; **448**: 30–39.
- 16 Morris HR, Masento MS, Taylor GW, Jermyn KA, Kay RR. Structure elucidation of two differentiation inducing factors (DIF-2 and DIF-3) from the cellular slime mould *Dictyostelium discoideum*. *Biochem J* 1988; **249**: 903–906.
- 17 Morandini P, Offer J, Traynor D, *et al.* The proximal pathway of metabolism of the chlorinated signal molecule differentiation-inducing factor-1 (DIF-1) in the cellular slime mould *Dictyostelium*. *Biochem J* 1995; **306**: 735–743.

- 18 Khoo KH, Maizels RM, Page AP, Taylor GW, Rendell NB, Dell A. Characterization of nematode glycoproteins: the major O-glycans of *Toxocara* excretory-secretory antigens are O-methylated trisaccharides. *Glycobiology* 1991; **1**: 163–171.
- 19 Masento MS, Taylor GW, Watson D, *et al.* Metabolism of 3-hydroxychrysene by rat liver microsomal preparations. *Chem Biol Interact* 1990; **74**: 163–178.
- 20 Wilson R, Sykes DA, Watson D, Rutman A, Taylor GW, Cole PJ. Measurement of *Pseudomonas aeruginosa* phenazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelium. *Infect Immun* 1988; **56**: 2515–2517.
- 21 Somerville M, Taylor GW, Watson D, *et al.* Release of mucus glycoconjugates by *Pseudomonas aeruginosa* rhamnolipid into feline trachea *in vivo* and human bronchus *in vitro*. *Am J Respir Cell Mol Biol* 1992; **6**: 116–122.
- 22 Scoppola A, Maher VM, Thompson GR, Rendell NB, Taylor GW. Quantitation of plasma mevalonic acid using gas chromatography-electron capture mass spectrometry. *J Lipid Res* 1991; **32**: 1057–1060.
- 23 Naoumova RP, Marais AD, Mountney J, *et al.* Acute and chronic effects on cholesterol biosynthesis of LDL-apheresis with or without concomitant HMG-CoA reductase inhibitor therapy. *Atherosclerosis* 1996; **119**: 203–213.
- 24 Naoumova R, Cummings MH, Watts GF, *et al.* Acute hyperinsulinaemia decreases cholesterol synthesis less in NIDDM than in non diabetic patients. *Eur J Clin Invest* 1996; **26**: 332–340.
- 25 Mazzone T, Foster D, Chait A. *In vivo* stimulation of low-density lipoprotein degradation by insulin. *Diabetes* 1984; **33**: 333–338.
- 26 Graham DY, Lew GM, Klein PD, *et al.* Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. *Ann Intern Med* 1992; **116**: 705–708.
- 27 Deprez PH, Ghosh P, Goodlad RA, *et al.* Hypergastrinaemia: a new mechanism. *Lancet* 1991; **338**: 410–411.
- 28 Lacey SL, Mehmet S, Taylor GW. Inhibition of *Helicobacter pylori* growth by 4-hydroxy-2-alkylquinolines produced by *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1995; **36**: 827–831.
- 29 Taylor GW, Machan ZA, Mehmet S, Cole PJ, Wilson R. Rapid identification of 4-hydroxy-2-alkylquinolines produced by *Pseudomonas aeruginosa* using gas chromatography-electron-capture mass spectrometry. *J Chromatogr B Biomed Appl* 1995; **664**: 458–462.
- 30 Vestal ML. Thermospray liquid chromatographic interface for magnetic mass spectrometers. *Anal Chem* 1984; **56**: 2590–2592.
- 31 Murray S, Rendell NB, Taylor GW. Microbore h.p.l.c. electrospray ionisation mass spectrometry of steroid sulphates. *J Chromatogr* 1996; in press.
- 32 Orie NGM, de Vries GA, Kikstra A. Growth of aspergillus in the human lung. Aspergilloma and aspergillosis. *Am Rev Respir Dis* 1960; **82**: 649–662.
- 33 Amitani R, Taylor G, Elezis EN, *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun* 1995; **63**: 3266–3271.
- 34 Allport J, Donnelly LE, Kefalas P, *et al.* A possible role for mono(ADP-ribosyl)transferase in the signalling pathway mediating neutrophil chemotaxis. *Br J Clin Pharmacol* 1996; **42**: 99–106.
- 35 Donnelly LE, Rendell NB, Murray S, *et al.* Arginine specific mono(ADP-ribosyl)transferase activity on the surface of human polymorphonuclear neutrophil leucocytes. *Biochem J* 1996; **315**: 635–641.
- 36 Hutchinson WL, Noble GE, Hawkins PN, Pepys MB. The pentraxins, C-reactive protein and serum amyloid P component, are cleared and catabolized by hepatocytes *in vivo*. *J Clin Invest* 1994; **94**: 1390–1396.
- 37 Tennent GA, Lovat LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proc Natl Acad Sci U S A* 1995; **92**: 4299–4303.