COLLEGE LECTURES

Medicine and the pharmacological revolution

The Harveian Oration of 1993

The Harveian Oration is given annually at the College under an indenture of William Harvey in 1656. The 1993 oration was given on 19 October by Sir Colin Dollery FRCP, Dean of the Royal Postgraduate Medical School, London.

Medicine without modern drugs would be of little value to patients. The progress of medicine has marched in time with pharmacology from its beginnings with natural products, through application of medicinal chemistry and quantitative pharmacology, up to the current impact of biotechnology. My lecture explores, very selectively, the historical background of innovations in drug therapy, including a brief review of successes and failures. It ends by posing an intriguing question: ‘If medicine without drugs is unthinkable, is the reverse necessarily true?’

William Harvey, the London Pharmacopoeia of 1618 and the College dispensary

In the early 17th century, the time of Harvey, Western medical treatment made its first hesitant steps to emerge from a past dominated by Hippocrates, Galen and the Arab physicians, notably Avicenna [1,2].

William Harvey was a much more interesting character than might be guessed from the severe expression in his portraits. Aubrey, in his Brief lives, gives a vivid account of Harvey’s character, noting that ‘He was hitt seated and in his young days would draw out a dagger upon very slight occasion’. Sadly, Harvey was not a model prescriber and drew the comment from Aubrey: ‘All of his profession would allow his to be an excellent anatomist, but I never heard of any that admired his therapeutique ways. I knew several practitioners in London that would not have given 3d for one of his bills (prescriptions): and that a man could hardly tell by one of his bills what he did aime at’; A few lines later he remarked, ‘He did not care for Chymistrey, and was wont to speak against them with an undervalue’ [3].

Was Harvey’s lack of interest in prescribing due to his realisation of the uselessness of most of the then current remedies or was his mind preoccupied with other matters? There do not appear to be any copies of Harvey’s prescriptions, or those of any other staff members from St Bartholomew’s, extant for the early 17th century. It was a time of expensive proprietary nostrums such as Scot’s pills, Goddard’s drops and Daffy’s elixir. A flavour of what prescribing was like can be had from, ‘Aqua Omnium Florum’, a favourite prescription of Dr Bates, who died in 1699. This was prepared from cow dung gathered in May, extracted in river water, mixed and digested for 24 hours. The clear liquid was decanted and consumed by the patient. The quack inventor of this nostrum made a fortune of £20,000 [4].

Although lists of formulae for medicines existed from the earliest times, there is little evidence of a systematic attempt to standardise the composition of these remedies until the mid 16th and early 17th century [5]. The first true pharmacopoeia appears to have been published under the authority of the Senate of Nuremberg in 1542. When Harvey studied in Padua in 1602, local ‘Antidotario’ were circulating in Italy but these were more in the nature of texts on pharmacy and therapeutics than true pharmacopoeias [6]. The London College was conscious of the need to bring some order to the chaotic prescribing situation in this city and its efforts culminated in the first pharmacopoeia in Britain, the Pharmacopoeia Londinensis, written in Latin and published in 1618 [7]. William Harvey was one of its sponsors. Among the 1,960 entries, there were few ingredients with useful therapeutical activity. Most were drawn from plant and animal sources, with a few metals and minerals. Common plant ingredients included Scylla; Myrrh; Crocus; Melissa [baum leaves]; Caryophylla [cloves]; Cardamom seeds, Nucis moschata [nutmeg]; Zingiber [ginger]; Squill; Gentian; Mallow, etc. Hope and magic were the main ingredients. The formula for Aqua Mirabilis in the 1618 pharmacopoeia is typical:

Caryophyllorum
Galangae
Cubebarum
Meliloti
Cardamoni
Nucis Moschatae
Zinziberis ana. drachma unam
Succi Chelidoniae libram dimidiam
Aqua vitae libram unam
Vini albi libras tres
Infundantur & distillentur per Alembicum

However improbable its contents, the London Pharmacopoeia of 1618 was the beginning of an attempt to standardise drug therapy, an essential precondition for proper study.

In those days the College was often in conflict with
the apothecaries and tension heightened after the great plague when most physicians left London with their wealthy patrons and the apothecaries remained behind to do their best for the dying. In 1696, perhaps to try to redeem its reputation with the populace, the College decided to establish a free dispensary for the poor in the coach house and stables of the College premises in Warwick Lane. The decision caused great controversy in the College. A famous battle took place between a Member of the College with his retinue and some of the College servants who dispensed the medicines. The battle was immortalised by Dr Sam Garth in his epic poem The Dispensary [8]. Garth conjures up a vivid image of an apothecary’s shop which makes the reader sympathetic to Garth’s mistrust of ‘chymestry’.

Here, Mummies lay most reverendly stale,
And there, the Tortois hung her Coat o’Mail;
Not far from some huge Sharks devouring head,
The flying fish their finny pinions spread.
Alot in Rows large poppy heads were strung,
And near, a scaly Alligator hung.
In this place, Drugs in musky heaps decay’d,
In that, dri’d bladders and drawn Teeth were laid.

The battle was an exciting affair as the Member and his retinue, who sympathised with the apothecaries, attempted to close down (or smash up!) the College dispensary. The temptation to quote from Garth’s splendid poem is irresistible.

Into the shop their bold battalions move,
And what their Chief commands the rest approve.
Down from the Walls they tear the shelves in haste,
Which, on their flanks for Pallisades are plac’d.

And now the Scouts the adverse Host decry,
Blue Aprons in the Air for Colours fly:
With unresisted Force they urge their way,
And find the Foe embattell’d in Array.
Then from their levell’d Syringes they pour
The liquid Volley of a missive Show’r

Like spouts in Southern Seas the Deluge broke,
And Numbers sunk beneath th’impetuous Stroak

And now the stag’ring Braves, led by Despair,
Advance, and to return the Charge, prepare
Each seizes for his Shield an ample Scale,
And the Brass Weights fly thick as showers of Hail
Whole heaps of Warriors welter on the Ground
With Gally-Pots, and broken Phials crown’d

Each combatant his Adversary mauls
With batter’d Bed-pan’s and stav’d Urinals.’

The battle ended when the invaders had the upper hand and their leader was about to plunge a knife into the chief defender. His life was saved when Apollo materialised in the shape of a fee and the member dropped his knife to grab at it.

The College was obviously an exciting place in those days but, apart from the London Pharmacopoeia which only began to be respectable with the 1746 revision, its contributions to the advance of medical treatment were not conspicuous.

The dawn of modern therapeutics: the fever trees

The fever tree from Loja (Loxa)

According to tradition, the second wife of the Spanish Viceroy in Lima, the Condesa de Chinchon, travelled by sea to Panama and then overland to Lima through the malarial valleys of Central and South America. She arrived in Peru in January 1629. En route she contracted a tertian fever which was unresponsive to treatment and it seemed that she would die. At her bedside in Lima an argument took place between her husband’s Jesuit confessor, Diego de Torres Velasquez SJ and the Viceroy’s personal physician, Juan de Vega. The confessor argued that she should be treated with an extract of the cinnamon coloured bark of the fever tree from Loxa, then in Peru, now in southern Ecuador [9]. Herphysician was unwilling to experiment with his high born patient but the Jesuit prevailed, it is said, after the Viceroy insisted that it was tried first on patients in public hospitals in Lima. Francisca Henriquez de Ribera, Countess of Chinchon, survived, possibly the first European to be cured of a life threatening disease by a drug, Cinchona was named (and misspelt) by Linnaeus after the Condesa although some believe an alternative derivation is from the Quechua (Inca) word kinia which means bark. The Countess died a few years later in South America and it was the Jesuits who introduced the bark into Europe.

The properties of the Peruvian bark seem to have been discussed ‘in the margins’ of a meeting of the 8th General Council of the Jesuits in Rome in 1645/6—perhaps the first international ‘medical’ congress! The Jesuits were the main importers, and sponsorship of the bark by Cardinal de Lugo SJ, an eminent philosopher, led to it being called ‘Polvis Cardinalsis’ in Rome. The Cardinal is said to have experimented on himself with the bark but he assigned the formal testing to Gabriele Fonseca, physician to Innocent X. De Lugo was so impressed by its properties that he established free dispensaries in Rome to dispense it because, as it had not been approved by Galen, the physicians were sceptical. In 1657 Fonseca successfully treated Flavio, Cardinal Chigi, nephew of Alexander VII with the bark [10]. Sir Robert Talbor was a pioneer of the use of Peruvian bark in England. He trained in medicine at Cambridge but probably learnt of the bark while apprenticed to an apothecary’s shop in that city before he took up his medical studies. He concealed the nature of his remedy and eventually sold his secret to
the King of France, Louis XIV. His treatise, entitled *The English Remedy*, published in 1682, was an anonymous translation from the French.

The Peruvian bark was first mentioned in the *London Pharmacopoeia* of 1676 and by 1746, the first edition in English, the following matter of fact description of its preparation was included.

Take one pound of peruvian bark, reduce it to powder. Water 10 or 12 pints boil for an hour or two. The liquor will be pellucid and red but turn yellow and turbid as it cools. Evaporate on a gentle fire until it attains a proper consistency.

It is difficult now to realise the enormous impact of the Peruvian bark upon European medicine. Malaria was endemic in northern Europe as well as south. Essex was referred to as a notoriously malarial county. Mortality was substantial and morbidity was high, and here for the first time was a drug which cured.

**The fever trees from Chipping Norton and Hoddesdon**

The Reverend Edmund Stone described the antipyretic properties of the bark of the white willow in a letter [11] to the Royal Society in June 1763 and this too has an interesting link with quinine. He described his discovery in these words: 'About six years ago, I accidentally tasted it and was surprised at its extraordinary bitterness; which immediately raised me the suspicion of its having the properties of the Peruvian bark'. After making a cautious dose-response curve with a pound of bark he had dried on the outside of a baker’s oven, he achieved great success in treating 50 patients with agues but failed in a few cases of autumnal or quartan agues. He added one fifth part of the Peruvian bark to it and ‘with this small auxiliary it totally routed its adversary’.

Stone’s paper, although often quoted now, attracted little medical interest at the time. A systematic evaluation of the bark of different species of willow was made by Samuel James in 1792 [12] who used the astringency of its taste, rather than the bitterness, to trace its antipyretic activity. Based on his data he estimated that 9 ozs of the broad leaved willow was equivalent to 24 ozs of the white willow bark. James also demonstrated that fevers that would not respond to the Peruvian bark would respond to the willow bark. The research was done so well that we might claim this unassuming young provincial surgeon to be among the first British clinical pharmacologists. A shortage of Peruvian bark in Europe caused by the English blockade during the Napoleonic wars led to renewed interest in the antipyretic properties of willow bark in the early 19th century and to the isolation of an impure form of salicylic acid, salicin, by Leroux in 1829 and further purification by E Merck in 1833. Exploitation of this discovery had to await advances in organic chemistry which led to the synthesis of salicylic acid by Kolbe in 1860 [13].

**Aniline dyes and the foundation of the pharmaceutical industry**

In 1856 WH Perkin, a young assistant to the first Director of the Royal College of Chemistry in London, AW von Hoffman conceived the idea that he might be able to synthesise quinine by oxidising allyltoluidine with potassium dichromate. The argument was simplistic and based on the known atomic composition of quinine:

\[
2\text{C}_{19}\text{H}_{24}\text{N}_3\text{O}_3 + 3\text{O} = \text{C}_{26}\text{H}_{24}\text{N}_2\text{O}_2 + \text{H}_2\text{O}
\]

Allyltoluidine Quinine

The experiment, carried out at home, did not work. Perkin knew that if quinine was treated with caustic soda, some aniline was formed so he tried again with aniline. That did not work either and he was left with a black precipitate at the bottom of his test tube. That black deposit was aniline mauve, the first synthetic diazo dyestuff and the precursor of the modern chemical industry. Perkin left to set up his own company but the theoretical understanding of the reactions involved in the synthesis of the aniline dyes was unravelled by the German chemists, and it was in Germany that much of the pioneering work was done up until the second world war [14]. The link with the aniline dyes is a thread that ran through the origins of the pharmaceutical industry. Observation of the antipyretic properties of acetanilide led Carl Duisberg, a chemist who later became director general of Bayer, to investigate a derivative of acetanilide, para-nitrophenol, which was produced in large quantities as a by-product of the synthesis of the diazo dyes. This in turn led to the production of phenacetin, the first relatively non-toxic analgesic, which enjoyed an enormous commercial success. For the first time the general population could cure their headaches. Attempts to produce a less gastric irritant form of salicylic acid led Felix Hoffman to synthesise aspirin. Knowledge that quinine contained a quinoline ring led Knorr to synthesise analogues containing this structure which evolved into antipyrene and later phenylbutazone. This, indeed, was the time when the organic chemist was king.

**In vivo testing**

German chemical science dominated the latter years of the 19th century and the early 20th century but there was also a rapid expansion of biomedical science with figures like Virchow in pathology and Koch in bacteriology. German scientists were also in the forefront of efforts to devise new drugs to treat infections. Much of this effort grew out of their ability to use dyes, a product of expertise in the dyestuffs industry, as stains to reveal features of micro-organisms. The concept evolved that if only bacteria could be stained in vivo, a way of killing them could be found. At this time sulphonamide groups were often linked to azo dyes in
order to confer colour fastness to textiles. Much unproductive effort took place until Gerhard Domagk [15] made the crucial decision to test candid dye in vivo rather than in vitro. His observation that the red dye prontosil would protect mice against a lethal injection of streptococci was a breakthrough. The paradox, revealed a short time later by the Trefouel, Nitti and Bovet from Fourneau’s laboratory at the Institut Pasteur in Paris, was that prontosil was split in vivo by azo-reductase to yield the colourless substance, sulphanilamide (162F), which was the active substance. This was the first documented example of an enzymically produced active metabolite. In current terminology prontosil is a ‘pro-drug’. Domagk’s contribution tended to be overlooked in England because it was made near the outbreak of war and Hitler would not allow Domagk to accept the Nobel prize for Medicine which was awarded in 1939. It was fortuitous that the French discovery was made by workers associated with the Rhone-Poulenc Pharmaceutical Company which then, as now, owned May & Baker in England. Barber’s group at May & Baker took up the study of the sulphonamides and synthesised sulphapyridine (through the slightly fortuitous chance that they happened to have some α-amino pyridine on the laboratory shelf). This was the famous M&B 693 [16], the first sulphonamide with high activity against pneumococci, studied in man by Lionel Whitby.

The sulphonamides are relatively simple chemical structures and an immense number of derivatives were synthesised [17]. They were quickly superseded as antibacterial agents by the beta-lactam antibiotics discovered by Fleming and developed by Florey and Chain [18] but the sulphonamide structure proved to have other important pharmacological properties. Some patients became hypoglycaemic during treatment, others developed a mild diuresis. Some of the compounds were also uricosuric. Using animal tests systems these activities were refined by skilful medicinal chemistry and led to the discovery of the sulphonylurea, tolbutamide. Exploitation of the diuretic activity led first to the carbonic anhydrase inhibitor, acetazolamide, and later to chlorothiazide and the multitude of other thiazide diuretics.

The marriage of medicinal chemistry and quantitative pharmacology

Close collaboration between a medicinal chemist and a pharmacologist using an accurate assay was (and still is) the basis of modern drug development. A skilled team can start with a lead compound and hope to improve its activity many fold, sometimes thousands of times, as they explore the chemical features that confboxer activity. It is a process which is being aided by computer modelling of both receptors and the agonists and antagonist molecules designed to interact with them. The example chosen here is the discovery of the antihistamines and the phenothiazine antipsychotics.

The origins of this approach can be traced back to the work of Henry Dale who had begun his career as a research pharmacologist at the Burroughs, Wellcome Company in 1904.

Dale’s research on fungus products, particularly ergot, led him to discover the biological activity and chemical structure (with Barger) of histamine. Dale and Laidlaw noted that histamine contracted smooth muscle in vivo but the response to an intravenous dose was much more complex and resembled anaphylactic shock. Dale also discovered the antagonist effect of ergot upon the pressor action of adrenaline [19].

Ernest Fourneau, a chemist who trained in Germany with Emil Fischer and became Director of Research at the Poulenc company and head of the Department of Therapeutic Chemistry at the Institut Pasteur in France, was one of the leading figures in the development of modern medicinal chemistry. He had already synthesised a number of phenolic ethylamines and ethers in 1910 in a search for better adrenaline antagonists. In 1937, Daniel Bovet, in collaboration with Fourneau, set out to discover an antagonist to histamine. Bovet and Staub chose first to screen some of the earlier phenolic compounds and they soon demonstrated in vivo antihistamine activity. One of these, 929F, protected a guinea pig against several times the lethal dose of histamine. Armed with a pharmacological assay, further series of compounds were investigated including aniline derivatives but all were too toxic in animals to use in man. They turned to Rhone-Poulenc for help where Halpern synthesised several active compounds including phenbenzamine (Antergan) and diphenhydramine (Benadryl).

Scientists at Rhone-Poulenc were interested in phenothiazines as potential antimalarials because of their structural resemblance to methylene blue which was known to have some anti-plasmodial activity. Paradoxically, if it had not been for the war, which cut off scientific contacts, the work would have been stopped, for the French workers would have learnt that these compounds did not have useful antimalarial activity. However, when some of the phenothiazine derivatives were submitted to their antihistamine bioassay, several, including phenothiazine and promethazine (Phenergan), proved to be highly active. The main side-effects of the antihistamines in man were sedation and tranquillity. Physicians and pharmacologists, at both Rhone-Poulenc and Merck in the USA began to wonder if this might be a useful property in its own right. Henri Laborit’s controversial work on antihistamines in surgical shock, anaesthesia ‘euphoric quietude’, and artificial hibernation also excited interest [20]. Starting in late 1950, Koetschet and his colleagues at Rhone-Poulenc, Paul Charpentier and Simone Courvoisier, using the rope climbing and maze test devised by Charles Winter at Merck, initiated a programme to devise phenothiazine compounds with greater central activity. Chlorpromazine (4560 RP) was amongst the first to be identified [21].
Initially, chlorpromazine was targeted for use in nausea and vomiting in pregnancy and for motion sickness. Delay and Deniker decided to try the drug against manic excitation at St Anne’s psychiatric hospital in Paris. Deniker credited his clinic nurse with first noticing that there was something very different about this new drug [22]. As Delay and Deniker extended their work they found good results in schizophrenia, particularly catatonia, a discovery that was to lead, in time, to a revolution in psychiatric care and the emptying of the lunatic asylums.

An entirely different line of enquiry was to lead Paul Janssen to the other main group of antipsychotics, the butyrophenones. His team were engaged in a successful attempt to separate the analgesic and constipating effects of codeine. Results included the antidiarrhoeal agent diphenoxylate and the powerful analgesic, dextromoramide. One new compound, R 1187, a butyrophenone derivative of nortriptyline, was not a strong analgesic but showed chlorpromazine-like activity, causing the animals to be calm, sedated and slightly catatonic. Further modifications of this structure guided by the pharmacological assay led to haloperidol [23].

In Britain, Sir James Black was to bring the marriage of precise pharmacological assay with medicinal chemistry to a high form of art. Little was left to chance in this hypothesis-driven, precisely targeted, approach which led to the discovery of the β adrenergic antagonist propranolol as a treatment for angina while he was at ICI, and the H2 receptor antagonist cimetidine for peptic ulcer after he moved to Smith, Kline & French.

The study of drugs in man

Compared with the rapid advance of experimental pharmacology and medicinal chemistry, systematic scientific study of drugs in man started slowly. However, such studies have made rapid progress in the last 30 years and have contributed greatly to understanding. In this account I shall consider three main developments.

1. Pharmacokinetics and metabolism.
2. Clinical pharmacology.
3. Randomised controlled clinical trials.

Pharmacokinetics

The early development of clinical pharmacokinetics has a link with quinine and the need for new antimalarials. During the second world war the US Army had large numbers of troops in the Pacific theatre exposed to malaria and thus there was an urgent need for new antimalarials. An extensive screening programme was initiated but there was the problem of how to test promising compounds in man with sufficient speed. To attack this problem Dr James Shannon brought together a group of investigators which included Dr Bernard Brodie at the Goldwater Memorial Hospital in New York. He insisted that the response should be related to plasma levels. Dr Shannon based this policy on two premises: (a) compounds that were inactive because the concentration was insufficient should be discarded as soon as possible, and (b) a knowledge of the physiological disposition of promising compounds was essential to help devise an optimum dosing schedule [24]. An important outcome of this programme was the realisation that the rates of metabolism of lipid soluble drugs varied very widely in healthy individuals.

The behaviour of lipid soluble drugs in the human body

Drugs used in oral treatment must be lipid soluble to allow them to pass across the enterocytes into the portal plasma (except for the few cases where they are actively transported). Once in the body, elimination by renal excretion is slow because lipid solubility allows the compound to diffuse back across the renal tubular epithelium as the urine is concentrated. Typically the renal clearance of a lipid soluble drug into urine approximates to the urine flow rate, not the glomerular filtration rate. High lipid solubility also allows the substance to enter body tissues, thus yielding a high apparent volume of distribution. A drug with a volume of distribution of only 70 litres and a clearance of 1 ml/min would have an inconveniently long half-life of about three half-days. Lipid solubility also allows ready entry into the hepatocytes and exposure to the cytochrome p450 family of enzymes, which can oxidise an extraordinary range of foreign chemicals, and to conjugating enzymes that can attach hydrophilic groups such as glucuronic acid. Oxidation is often followed by conjugation. The amounts of different p450 isoforms in the liver vary greatly in different normal people and contributes to a very wide range of plasma concentrations in patients taking the same dose. The work of Alexanderson and Sjöqvist showing a twenty-fold range in steady-state plasma concentration of plasma nortriptilin in depressed patients all taking 75 mg of the drug daily, is a striking but by no means unique example [25].

One of the great contributions of pharmacokinetics has been to provide a rationale for linking animal pharmacology and toxicology with studies in man through the common medium of the plasma concentration [26]. Many of the phenomena which can be demonstrated in animal pharmacology and toxicology are relevant to human use only under conditions of gross overdose. The membrane stabilising action of propranolol is an example. Pharmacologists interested in the anti-arrhythmic action of propranolol believed that this ‘Class 1’ effect made an important contribution to the action of the drug. The concentration required for the membrane effect exceeded 1 microgram per ml, whereas the concentrations achieved in
ordinary therapeutic use rarely exceeded 100 nanograms per ml. For some drugs that have a long half life in man and a very short one in animals such as the rat, the ‘margin of safety’ based on the lowest toxicity free dose in the animal compared with the therapeutic dose in man gave false comfort. A classical experiment showed that the concentrations of phenylbutazone which caused severe gastrointestinal ulceration in the dog were similar to the therapeutic concentrations in man although the doses in dogs required to achieve that concentration were much higher. In this case the safety margin, apparently demonstrated in animal toxicity testing, was illusory.

Metabolism

The reason why most lipid soluble drugs have a shorter duration of action than would be predicted from their physical properties is that they are metabolised in the liver, predominantly by the cytochrome p450 family of enzymes, to more water soluble derivatives. The p450s are a super family of membrane bound haemoproteins [27]. They utilise molecular oxygen and NADPH to oxidise a wide range of endogenous and exogenous compounds. More than 20 different p450s in four main families have been identified in the human liver but the total number of isoforms in different tissues is substantially higher. Several of the hepatic isoforms show important genetic polymorphisms [28]:

- CYP1A2 catalyses the o-de-ethyllylation of phenacetin and is dependent in 10% of Caucasians;
- CYP1IIC family. Polymorphisms of oxidation of mephenytoin in 14–22% of Chinese and 3–6% of Caucasians;
- CYP1ID6. About 8% of a Caucasian population do not express this enzyme which metabolises a number of clinically important drugs such as codeine, metoprolol, encainide and propafenone;
- CYP1IIB5 is expressed in only about 25% of people. It metabolises dihydropyridines and other drugs.

Drugs are often substrates for more than one isoform and, if they are, deficiency or inhibition of one form has only a limited effect upon the overall rate of metabolism. If metabolism proceeds predominantly via a single isoform, the individual is vulnerable if he/she has a genetic deficiency of that form or takes another drug that inhibits it. For example, patients with normal amount of CYP1ID6 convert about 4% of a dose of codeine to morphine while those who are deficient in the enzyme convert less than 0.3%. CYP1ID6 is inhibited by low doses of quinidine and concurrent administration of this drug with encainide or propafenone can lead to sharply increased plasma concentrations of these drugs. The antihistamine terfenadine, which prolongs the QT interval of the ECG, is subject to extensive presystemic metabolism by CYP1IIB5. Concurrent use of ketoconazole, which is a potent inhibitor of CYP1IIB5, has raised plasma terfenadine from < 7 ng/ml to > 80 ng/ml with lengthening of the mean QT, from 416 to 490 msec [29]. Fatalities from the ventricular arrhythmia, torsades de pointes, have been reported following this interaction.

Kinetics and metabolism has attracted relatively little attention from clinicians apart from a few well publicised examples such as drug interactions due to inhibition or induction of metabolism with warfarin and problems with drugs mainly excreted unchanged by the kidney, such as digoxin and aminoglycoside antibiotics, in patients with renal failure. Its importance as a source of understanding of individual differences in response has found limited applications in therapeutic monitoring of plasma concentrations, particularly of anti-epileptics and cardiac anti-arrhythmics. The desire for simplified dosage regimens has led most physicians to ignore individual variations in kinetics as a source of unsatisfactory therapeutic response or avoidable toxicity.

Clinical pharmacology

Cardiovascular drug therapy, especially of hypertension, was the catalyst of modern clinical pharmacology. Drugs that lowered blood pressure or altered heart rate provided easily measurable indices of drug response and the first attempts to construct accurate dose-response curves and estimates of duration of action. It was a short step to using challenges such as exercise or the administration of an agonist or an antagonist to assess responses which were difficult to measure in the resting physiological state. Studies of the inhibition by propranolol of exercise or isoprenaline-induced tachycardia excited many clinical pharmacologists. As this drug also had a high presystemic clearance in the liver and an active metabolite (4-hydroxy-propranolol) it offered a range of interesting, even distracting, opportunities [30].

Studies of this kind have become quite sophisticated, using agonists and antagonists to probe each other’s properties. Clonidine is an a2 adrenoceptor agonist which is highly lipid soluble and penetrates the blood-brain barrier readily. I have chosen its study as an example from our own work. A 200 microgram intravenous dose of this substance in a normal man causes a rapid onset of sedation, a fall in blood pressure and heart rate accompanied by reduced sympathetic activity and a sharp reduction in salivary flow. The blood pressure reduction and bradycardia can be measured by standard methods. A fall in plasma noradrenaline concentration can be used as an index of the decline in sympathetic activity. Salivary flow can be measured using preweighed dental cotton wool rolls [31]. Clonidine also stimulates an a1 adrenoceptor on pancreatic islet cells causing a small reduction in insulin output and a rise in blood sugar.

Alpha2 adrenoceptor antagonists have been developed for two potential therapeutic indications. One
concept has been to use them as central stimulants in depression by analogy with the awakening action of yohimbine. The other has been to develop compounds that do not pass the blood-brain barrier but which can be used to stimulate insulin release from the pancreatic islet cells. The ability of these drugs to antagonise various actions of intravenous clonidine has been a valuable method of assessing their pharmacological action in man. A 2mg dose of MK-912, an α₂ antagonist which enters the brain, almost completely inhibited the clonidine-induced hypotension, bradycardia, xerostomia, and increase in plasma glucose concentrations that were observed during the placebo treatment period [32]. MK-467, an α₂ antagonist which scarcely enters the brain, did not modify the clonidine-induced decrease in blood pressure or heart rate. However, the clonidine-induced increase in blood glucose and suppression of plasma insulin were inhibited by MK-467 [33]. These studies provide an example of our ability to answer precisely formulated pharmacological questions in man.

Clinical trials

Fortunately for their inventors, a number of the early drug discoveries produced such dramatic short-term effects that there was little room for doubt about their efficacy. Examples include aspirin for headache, sulphaspyridine in pneumococcal pneumonia, hexamethion in malignant hypertension, and vitamin B₁₂ in pernicious anaemia. But many potentially useful remedies have actions that are more subtle or manifest over a longer period of time. Judging their efficacy purely by observation of small numbers of patients over a short time is virtually impossible.

The concept that trials of new forms of treatment are an experiment that must be properly controlled was slow to be accepted. Armitage [34] has pointed out that ‘alternation’ was used in therapeutic trials of a serum against diphtheria by Fibiger in 1898 and ‘randomisation’ was used in psychology experiments by Peirce in 1884, but these pioneer efforts had no lasting impact. The importance of random assignment was expounded by R A Fisher [35] in 1935 but this was in respect of agricultural, rather than medical, experiments.

The need to evaluate the efficacy of streptomycin in tuberculosis gave the impetus for modern clinical trials and much of the credit must go to Austin Bradford Hill [36] who was the statistician to the MRC trial programme starting in 1946. There had already been a long history of drugs that showed some initial promise in the treatment of tuberculosis but later proved valueless. The need for rigour was reinforced by the shortage of streptomycin. Bradford Hill and his colleagues on the Tuberculosis Trials Committee designed the first true randomised controlled trial [37] using sealed envelopes containing either ‘C’ for control or ‘S’ for streptomycin. The trial only included 107 patients, each followed for six months. At the end of that time the result was decisive, four of 55 patients given streptomycin and 14 of 52 controls were dead (p < 0.01). Forty years later, the MRC Hypertension Trial [38] screened nearly half a million people to recruit about 18,000 individuals into a trial lasting five years to demonstrate conclusively that lowering mild hypertension approximately halves the incidence of stroke.

Randomised controlled clinical trials have become an indispensable component of therapeutic advance. They range in size from groups of 10 or 20 used in studies of the clinical pharmacology of new therapeutic agents, up to tens of thousands in outcome trials, particularly in cardiovascular disease. Large outcome trials have such power and beauty that it is easy to be blind to their limitations, but there are some. Strict exclusion criteria may make results difficult to generalise. Large size, and a simple protocol means little or no data about therapeutic dose-response. Intention to treat analysis in a study with many withdrawals from randomised treatment may reduce the chance of detecting a pharmacological effect that requires the presence of the drug.

Experimental designs have been proposed that address some of these problems. Elimination of almost all exclusion criteria makes the results easier to generalise at the expense of more variation in the original data. Adjusting the dose to achieve a target plasma concentration in a ‘concentration controlled trial’ has been proposed as a means of eliminating pharmacokinetic variability and as a way of constructing a therapeutic concentration-effect curve. Neither of these concepts has yet been widely tested.

The pharmacology of proteins

The targets of most of these drugs are proteins and the idea that proteins might themselves be used as drugs is not novel; insulin has been in use for many years. However, the widespread use of pure proteins as therapeutic agents is a product of the molecular revolution. Molecular biology has been responsible for two major advances in pharmacology: (a) the use of biotechnology to produce pure proteins to use as therapeutic agents, and (b) the dissection of the human genome which has revealed large numbers of sub-types of receptors and enzymes which are potential drug targets.

Biotechnology and protein drugs

The work of Cohen and Boyer in 1972/73 with bacterial plasmids led to the concept of using self-replicating plasmids for gene cloning. By joining together lengths of DNA in vitro and integrating these into plasmids they introduced recombinant DNA technology. By 1978 they had succeeded in expressing the mouse dihydrofolate reductase gene in E.coli [39] and the foundations of the biotechnology industry which led
to companies like Genentech had been laid. It is now possible to express human genes in E. coli or yeast cells and thereby produce large quantities of a desired protein. The first targets were hormones such as insulin and growth hormone. Recombinant techniques came in the nick of time when fears about the safety of material isolated from human cadavers had been aroused by the incidence of Creutzfeldt-Jakob disease in patients with hypopituatism who had been treated with human growth hormone made from large batches of pooled human pituitaries. The availability of human insulin made by biotechnology has averted a looming shortage of insulin from animal pancreases. The isolation of the genes for blood cell growth factors led first to erythropoietin and then to GM-CSF and G-CSF. It does not seem fanciful to suppose that specific growth factor(s) will eventually be cloned and expressed for every tissue in the body.

Many cytokines have been cloned and expressed and a number have found therapeutic uses, although thus far for special indications in small numbers of patients with cancer. Not far behind there are a host of humanised antibodies under investigation, mainly by small biotechnology companies. These use gene expression techniques to produce antibodies in which the complementarity determining regions are derived from a mouse antibody raised against the target molecule but the rest of the antibody molecule is human. This technique produces antibodies with a very low immunogenicity but often also a relatively low affinity. The first of these products to achieve commercial prominence, Centoxin an anti-endotoxin antibody, proved to be disappointing in therapeutic use.

Among the more important biotechnology products which have already been marketed are the following.

| Hormones | Growth factors | Coagulation factors |
|----------|----------------|---------------------|
| Somatropin | Epoietin | Factor VIII |
| Insulin | Filgrastim | Factor IX |
| GMCSF | | |

| Cytokines | Enzyme | Antibody |
|-----------|--------|----------|
| Interferon-alpha | Alteplase | Centoxin |
| Interferon-gamma | | |
| Interleukin-2 | | |

The hormones, growth factors and coagulation factors are mainly used in physiological replacement therapy and represent a remarkable advance in therapeutics which owes little to classical pharmacology. Alteplase (TPA) was a great achievement but one that was somewhat overshadowed by the rediscovery of the value of the much cheaper streptokinase. Centoxin has been withdrawn but many other humanised antibodies are being investigated. The number of proteins under development by biotechnology companies, mainly in the USA, is staggerine. How many will fulfil the expectations of the venture capitalists who have flocked to back them?

*Proteins make lousy drugs*

This comment which was attributed, possibly apocryphally, to a research scientist at Merck & Co in the USA, has more than a grain of truth. The pharmacokinetic properties of proteins are a major limitation. These bulky hydrophilic molecules are degraded by digestive enzymes and have great difficulty in crossing cell membranes or epithelial surfaces with tight junctions. Although much ingenuity has been exercised to try and provide reliable oral dosage forms of peptides and proteins, these efforts have had only very limited success. Treatment with proteins normally involves parenteral, not enteral, treatment. Proteins do not normally cross the blood-brain barrier and enter cells with difficulty unless there is a specific mechanism for internalising them.

If the disease is life threatening (eg diabetes) or disabling (hypopituitary dwarfism) the inconvenience of prolonged treatment by injection is readily accepted. If the protein is itself the drug, for example replacement of deficiencies of circulating coagulation factors, there is little alternative. But proteins are unsuitable for prolonged use in less serious diseases and impractical for prophylaxis or prevention (apart from vaccines). Is there an answer to this dilemma? One possible approach has sprung from another advance of molecular science, the cloning of many human receptors and enzymes.

*Receptors galore*

In the early days of modern pharmacology it was thought that there would be a single unique receptor for each agonist molecule. Soon it became clear that the situation was more complex. The responses of different tissues to structurally related agonists and antagonists all acting upon the same ‘receptor’ type varied markedly. The example I have chosen to illustrate is the adrenergic receptor.

Dale first showed that adrenergic responses varied in their sensitivity to antagonists such as ergot alkaloids. Ahlquist [40] developed more robust evidence for different subtypes of adrenergic receptors by demonstrating different rank orders of potency in different tissues and designated these as alpha and beta. Lands [41], using a very similar technique to Ahlquist, was able to separate two subclasses of the beta receptor, β₁ and β₂. Relatively selective agonists and antagonists to these subtypes soon became available. Evidence for a distinct pre-junctional alpha receptor led Langer [42] to subdivide this receptor into the post-junctional α₁ and the pre-junctional α₂. Potent α₁ and α₂ agonists and antagonists were soon discovered. One surprising result was that α₁ agonists, eg methoxamine, raised blood pressure but α₂ agonists, eg clonidine, lowered it, by causing central inhibition of the sympathetic outflow.

With a wide range of agonists and antagonists avail-
able, many of them radiolabelled, it became obvious that the situation was even more complex and further subdivisions of both types of alpha receptor and a third beta receptor were proposed.

At this stage gene cloning techniques disclosed many additional candidates for adrenergic receptor subtypes and reconciliation of the pharmacological and genomic classification has become a major challenge. I have chosen to illustrate this with the \( \alpha_2 \) adrenergic receptor which I mentioned earlier in relation to our clinical pharmacological work.

**Alpha\(_2\) receptors**

On pharmacological grounds four subtypes have been proposed. These are designated \( \alpha_{2A}, \alpha_{2B}, \alpha_{2C}, \alpha_{2D} \). The initial division into \( \alpha_{2A} \) and \( \alpha_{2B} \) was made on the basis of relative affinity for the \( \alpha_1 \) ligand, prazosin, for which \( \alpha_{2B} \) had high affinity and \( \alpha_{2A} \) low affinity. Studies of the ability of various antagonists to inhibit binding of tritiated rauwolscine have led to two additional subtypes, \( \alpha_{2C} \) and \( \alpha_{2D} \) being proposed, although distinguishing \( \alpha_{2A} \) and \( \alpha_{2B} \) poses difficulties. The properties of a number of novel antagonists developed by Ruffolo’s group [43] at Smith, Kline and French suggest that there may be yet further subtypes of pharmacologically distinct \( \alpha_2 \) receptor. Three human \( \alpha_2 \) subtypes have been cloned and are identified at present by their chromosomal location as \( \alpha_{2C2}, \alpha_{2C4}, \alpha_{2C10} \) [44, 45]. The pharmacological \( \alpha_{2A} \) and the cloned \( \alpha_{2C10} \) appear to be identical and correspond to the human platelet \( \alpha_2A \) receptor. Alpha\(_{2C2}\) and \( \alpha_{2C4} \) may correspond but at present there are still significant discrepancies between the functional, radioligand and cloning studies.

**Simplicity or complexity?**

When I first joined the International Union of Pharmacology (IUPHAR) receptor nomenclature group I cherished the simple belief that cloning would solve all the problems and it would be possible to pair off the cloned genes with functionally defined receptors. The confusion prevailing with the \( \alpha_2 \) receptor subtypes may be simply that there is more cloning to be done and more attention needs to be paid to comparisons of the properties of expressed genes and intact tissues from the same species. But it seems undeniable that there are many more subtypes than originally suspected and this story has been repeated in almost all other receptor types investigated (eg acetylcholine, serotonin etc.). The reasons are perplexing. Why so many receptors for, in this case, a pair (adrenaline and noradrenaline) of agonists? One possibility is to achieve a graded response in different adjacent cells, an explanation which may have some credibility for adjacent neurones in the brain. Another suggestion is that a finite number of promoter sequences is possible for any one gene and different subtypes evolved in different cells utilising different promoters. But sometimes a single cell appears to express more than one subtype which is even more difficult to understand. But irrespective of the explanation, the galaxy of opportunities for developing agonists and antagonists that are more specific because they are selective for a subtype which is expressed in a particular target tissue offers a great incentive for the pharmacologist and medicinal chemist.

**High throughput screens**

Drug hunters must have a starting point. In the early days of pharmacology, natural products often provided a lead whose chemical structure could be determined and then modified to produce synthetic agonists and antagonists. Observations of unexpected responses in man have also been an important source of clues to new kinds of biological activity, for example with the sulphonamides. Much of modern drug discovery has been based on accurate and specific bioassays using living tissues in organ baths, combined with the skill of the medicinal chemist who made progressive modifications of the structure. This approach has proved much more difficult to apply to receptors whose natural agonists are peptides and proteins. Peptides are flexible molecules whose spatial configuration during receptor interaction is not accurately known. The receptor-interacting groups often lie on one face of an alpha helix and substitution of single amino acids frequently leads to complete loss of biological activity. The challenge has been to find non-peptide structures that will interact with the peptide receptor in the same way that morphine interacts with encephalin/endorphin receptors.

The strategy adopted to achieve this objective has been to utilise automated high throughput screens, some of them capable of screening thousands of compounds a week. These screens utilise eukaryotic cells which express a single human receptor subtype and are based on replacement of a radiolabelled ligand. The chemicals chosen for screening are drawn from repositories of substances synthesised in previous research programmes. Chemical and pharmaceutical companies have tens of thousands of such compounds in their files. If the screen records a ‘hit’ the medicinal chemists and pharmacologists get to work on the new lead to enhance its activity. Pharmaceutical companies are secretive about the details of these assay systems for commercial reasons, but there appear already to have been successes. The non-peptide antagonists of cholecystokinin A and B receptors seem to have originated from such a programme, in which the initial biological activity was increased many thousand fold by chemical manipulation [46].

It is too early to say what success these programmes may have but automation of assays, possibly coupled to similar automation of syntheses, promises to speed up
the search for new leads which is the rate limiting step in the introduction of really novel therapeutic agents. Their value in medicine will depend upon the significance of the control mechanism concerned in the pathogenesis of a disease process. Critics fear that the result may well be large numbers of drugs in search of a disease to treat.

Success and failures
Pharmacology and medicinal chemistry have transformed medicine from an intellectual exercise in diagnosis into a powerful force for the relief of human disease. What can be achieved today would have seemed little short of miraculous to a physician or a patient in Harvey’s time. But it is important not to exaggerate for many of the drug discoveries, although very worthwhile, are far from ideal. There are three major problem areas: (a) drug targeting, (b) individualisation of dose, and (c) degenerative diseases.

Targeting
Drugs are stupid although the neurotransmitters, cytokines and growth factors they emulate or antagonise are smart. The difference lies in the precision of targeting. Endogenous substances are usually released only in the immediate vicinity of cells they are designed to stimulate. Efforts to improve drug targeting have relied chiefly upon local administration. Various ingenious ideas such as antibodies linked to drugs have had little success, even in oncology. In principle, it ought to be possible to devise drugs with dual affinities that can achieve greater specificity because they bind to a surface molecule which is characteristic of the target cell type even if the target receptor is not confined to it. In practice, no-one has yet achieved this. Apart from skill in designing drugs that will or will not enter the CNS, based mainly on their lipid solubility, specific drug targeting remains a dream. Conceivably it is a dream that will be realised using gene transfer techniques with tissue specific promoters, but that is for the future.

Individualisation of dose
If drugs are stupid many of those who prescribe them are not very skilful. The ideal new drug for a pharmaceutical company representative to promote is one that can be prescribed as one tablet once a day. Both patients and their physicians prefer this simple approach. Yet most drugs are metabolised and the individual exposure to the drug in that single tablet often varies over a range of several fold. Much greater effort to optimise the treatment of individuals is needed.

A scoresheet
Some forms of drug treatment are more effective than others and I have attempted to classify the results of treating some common diseases on a scale of 1 to 5.

Class I. Restoration of normality.
a Replacement therapy of physiological deficiency
b Chemotherapy of most bacterial infections

Class II. Close approach to normality in many patients
a Analgesia/anaesthesia
b Angina
c Asthma
d Epilepsy
e Hypertension
f Peptic ulcer
g Thrombo-embolism

Class III. Substantial amelioration with continuing disability
a Acute myocardial infarction
b Depression
c Diabetes
d Many lymphomas
e Parkinson’s disease

Class IV. Symptomatic relief with residual major disability
a Heart failure
b Schizophrenia
c Rheumatoid arthritis
d Most virus diseases

Class V. Trivial or minor improvement
a Most dementias
b Many carcinomas
c Osteoarthritis
d Stroke

One obvious feature of the failures is that many involve degenerative changes in vital organs such as heart, brain and joints. If drugs are ever to address those problems they will need to be used in a much more sophisticated fashion than anything we can imagine today. Yet many diseases look ripe for progress, notably common cancers and CNS diseases such as depression and schizophrenia. The future of pharmacotherapy will be full of excitement.

A teasing finale
Doctors need drugs but do drugs need doctors? Could the pharmacological revolution ultimately cast aside the physicians? Many patients already regulate their own medicines rather skilfully after an initial diagnosis and medical advice. Many simple remedies are available over the counter in pharmacies and their numbers will grow as patents expire on relatively safe medicines like ibuprofen. Large research-intensive pharmaceutical houses are buying up generic manufacturers and suppliers of inexpensive drugs direct to patients.

The challenge is for physicians to develop greater skill and understanding in the use of therapeutic
of genetic and environmental factors. Ann NY Acad Sci 1971;178:739–51.
26 Davies DS, Prichard BNC (eds). Biological effects of drugs in relation to their plasma concentrations. London: Macmillan, 1973.
27 Guengerich FP. Characterisation of human cytochrome P-450 enzymes. FASEB 1992;6:745–8.
28 Kalow W (ed). Pharmacogenetics of drug metabolism. New York: Pergamon Press, 1992.
29 Honig PK, Worthing DC, Zamani K et al. Terfenadine ketoconazole interaction. Pharmacokinetic and electrocardiographic consequences. JAMA 1993;269:1153–8.
30 Dollery CT, Paterson JW and Conolly ME. Clinical pharmacology of beta receptor blocking drugs. Clin Pharmacol Ther 1969;10:759–65.
31 Dollery CT, Davies DS, Draffan GH et al. Clinical pharmacology and pharmacokinetics of clonidine. Clin Pharmacol Ther 1976;19:11–7.
32 Warren JB, Dollery CT, Fuller RW et al. Assessment of MK-912, an alpha-2 adrenoceptor antagonist, with use of intravenous clonidine. Clin Pharmacol Ther 1989;46:103–9.
33 Warren JB, Dollery CT, Sicberries D, Goldberg MR. Assessment of MK-467, a peripheral alpha-2 adrenergic receptor antagonist, with intravenous clonidine. Clin Pharmacol Ther 1991;50:71–7.
34 Armitage P, Bradford Hill and the Randomized Controlled Trial. Pharm Med 1992;6:23–57.
35 Fisher RA. The design of experiments. Edinburgh: Oliver & Boyd, 1935.
36 Hill AB. Principles of medical statistics. Lancet 1937.
37 Medical Research Council. Streptomyacin treatment of pulmonary tuberculosis: a report of the Streptomycin in Tuberculosis Trials Committee. Br Med J 1948;2:769–82.
38 Medical Research Council Working Party. MRC trial of treatment of mild hypertension: principal results. Br Med J 1985;291:97–104.
39 Chang ACY, Nunberg JH, Kaufman RJ et al. Phenotypic expression in E colii of a DNA sequence coding for mouse dihydrofolate reductase. Nature 1978;275:617–24.
40 Ahlquist RP. A study of adrenotropic receptors. Am J Physiol 1948;155:368–600.
41 Lands AM, Arnold A, McAuliff JP et al. Differentiation of receptor systems activated by sympathomimetic amines. Nature 1967;214:597–8.
42 Langer SZ. Presynaptic regulation of catecholamine release. Brit J Pharmacol 1974;60:481–97.
43 Ruffolo RR, Nichols AJ, Stadel JM, Hieble JP. Structure and function of alpha-adrenoceptors. Pharmacol Rev 1991;43:475–505.
44 Regan JW, Kobilka TS, Yanf-Feng TL et al. Cloning and expression of a human kidney cDNA for an alpha adrenergic receptor subtype. Proc Nat Acad Sci USA 1988;85:6301–5.
45 Link R, Daunt B, Barsh G et al. Cloning of two mouse genes encoding alpha-adrenergic receptor subtypes and identification of a single amino acid in the mouse alpha2C10 homolog responsible for interspecies variation in antagonistic binding. Mol Pharmacol 1992;42:216–27.
46 Evans BE, Bock MG, Rittle KE et al. Design of potent orally effective, non-peptidal antagonist of the peptide cholecystokinin. Proc Nat Acad Sci USA 1986;83:4918–22.

Address for correspondence: Sir Colin Dollery, Office of the Dean, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN.

The Harveian Oration is published in booklet form and is available from the College, price £3.00.