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SPECIAL LEAD ARTICLE

Reflections on drug research

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Part 1

Empirical approaches, discovery and invention

One of the more striking characteristics of human civilization has been his attempts to use his intelligence to seek relief from his various bodily ailments. His original efforts were entirely empirical like a game of 'Blind Man's Buff'. There is a record of these early activities in a museum in Heidelberg which authentically recreates the official pharmacies of folk-lore medicine that operated in Germany from the 14th to the 18th centuries. In their day, these pharmacies administered the official pharmacopoeias, the currently accepted list of remedies and nostrums of the day. The museum shows how the pharmacopoeias kept changing. As products fell into disfavour, new products appeared to fill the gaps. We now know that many diseases will ameliorate for a time by giving biologically inert-substances – placebos. So, from a therapeutic point of view, were these drugs intrinsically useless? Probably most of the remedies were indeed worthless. But folk medicine had its successes; some of them are still used in modern medicine. The roots of the belladonna plant are a source of atropine, the leaves of the foxglove are a source of digitalis, and the seeds of a common poppy provide morphine. How people originally found out about the therapeutic properties of these plants is truly a mystery.

This empirical approach to therapeutics based on trial and error did not give way to an empirical one based on scientific knowledge until the middle of the 19th century. In 1856, William Perkin, then 18 years old, was doing amateur chemistry experiments on the top floor of his parent's home in London (Harrow, 1921). At that time, London was heated and lit by gas derived from the distillation of coal, a process that produced by-products, including coal tar. Professor Hofmann, Perkin's tutor, had shown him how naphtha extracted from coal tar could be converted to the artificial alkaloid known as naphthalidine, and Perkin was trying to convert this artificial alkaloid into a drug, the natural alkaloid, quinine. Quinine had come to Europe from South America in the 17th century where its antimalarial properties had been discovered by folk medicine. It was in short supply and very expensive but vitally important to prevent malaria which was rampant in many parts of the world. Perkin's experiment failed but he did produce a magnificent new colour – mauve, the first aniline dye. Mauve led to a whole new industry for synthesizing artificial dyestuffs and thence to the development of explosives, perfumes, photography and modern therapeutic drugs.

After Perkin's experiment, the next contribution of dyestuffs to medicines came from Paul Ehrlich who was born 2 years before Perkin made his great discovery. By the time Ehrlich went to university, he had developed a passion for the microscopy of animal tissues, mounted on glass slides and stained with aniline dyes. Although he was not the first to use aniline dyes to stain tissues, he was the first to develop a theory on the staining process. He graduated in medicine when he was 24 and his graduation thesis on 'On the theory and practice of histological staining' was remarkable. His early ideas about the chemical affinity of dyes would lead him on to his work with von Behring on antitoxins and his concepts of serum therapy. Due to his work on antitoxins he developed his ideas about receptors (Maehle *et al.*, 2002). He imagined that cells sprouted finger-like processes that, with specific receptors expressed on their surfaces, could bind toxin molecules on a one-to-one basis. This receptor idea turned out, years later, to be the basis for the invention of many new drugs. Then he discovered that living cells in tissue culture, as distinct from pieces of dead tissue fixed in blocks of wax, could be selectively stained by another dyestuff, methylene blue. From this, he developed the idea of chemotherapy as a concept-driven goal. What he wanted was to find dyestuffs, like methylene blue which would selectively bind to receptors on the surface of living cells but which, unlike the blue dye, would be toxic and would kill the cell. He knew that many

dyestuffs were organic compounds of the well-known poison arsenic. He therefore set out to screen organic arsenicals for treating rabbits infected with the spirochete that had recently been discovered to be the cause of syphilis and, incredibly, he succeeded! He first showed that the structure attributed to atoxyl, an organic arsenical and a highly toxic parasiticide, was wrong – in chemical jargon, he proved that it was not the non-reactive anilide that everyone supposed but a chemically reactive amino-phenyl-arsenic acid and thus available to make derivatives. Starting from this, he systematically changed its structure using chemical ideas that he had developed in a lifetime's study of the chemistry of dyestuffs. Each new compound was injected into rabbits infected with the syphilis parasite. If there was no cure, then the next compound was tested. If there was a cure, then he wanted to know if the curative dose was much smaller than the toxic dose, a ratio he defined as the therapeutic Index. Eventually, in 1910, he found a substance called dioxy-diamino-arseno-benzene that was highly effective against experimental syphilis and had a good therapeutic Index (Lloyd *et al.*, 2005). It was the 606th compound that he had synthesized and tested and became famous as the drug known as Salvarsan. Ehrlich was truly the father of modern medicinal chemistry, and also of the modern pharmaceutical industry.

Domagk was another pioneer. While still a medical student, he joined the German army in 1914 and saw at first hand the helplessness of doctors faced with cholera, typhus, gas gangrene and infectious diarrhoea. He completed his medical degree after the war and became an academic pathologist and bacteriologist. In 1927, he joined I.G.Farbenindustrie, a manufacturer of azo-dyes, as Research Director, and was inspired by Ehrlich to test their dyes against haemolytic streptococcal infections in mice. He followed Ehrlich's example of 'synthesise new compound, test in mice – if negative, synthesise modified compound and test again'. In this way, he discovered the bacteriostatic effects of Prontosil Red but was uncertain that a compound that was so effective against streptococcal infections in mice would also be effective in man. Then, his daughter fell seriously ill with a streptococcal infection. In desperation, he treated her with Prontosil and she made a complete recovery. He told no one about this reckless experiment until the results of human studies were announced in 1937. Prontosil Red was the forerunner of sulphonamides and the chemotherapeutic revolution (Grundmann, 2006).

The most prolific drug discoverer to date has been Paul Janssen, a Belgian doctor who established his own drug company, and began his research in 1945 after the war by starting from pethidine, a drug that had been introduced into medicine in 1939 as an antispasmodic agent for relieving diarrhoea and colic. Pethidine was also found to have morphine-like properties and because of its addictive properties could no longer be prescribed for its antispasmodic activity. Janssen wanted to see if he could separate the two properties. Pethidine is a piperidine derivative and he knew that piperidine chemistry was fairly easy, so he proceeded systematically to modify its structure and evaluate the properties of the new molecules using two simple tests. The first test, known as the Straub Test, is when a mouse is injected with an opiate and its tail goes erect. In the second test, a piece of intestine, isolated in warm salt solution in a small bath, is stimulated electrically. The tissue contracts but when an antispasmodic compound is added to the bathing fluid, these contractions are suppressed. By systemically synthesizing and testing new compounds using these two tests, he gradually built up a picture of the specific chemical structures needed for the two properties – we now call them 'structure-activity relations' and they are at the heart of modern medicinal chemistry. Eventually, he succeeded by inventing two new drugs. These were 'fentanyl', a potent morphine-like analgesic, and 'loperamide' (Diocalm) a potent antispasmodic and antidiarrhoeal agent. Both are still highly successful drugs today. Just as Ehrlich's head, from years of experience, was full of an array of chemical groups that he referred to as chromophores, so Dr Paul, as his researchers knew him, had his head full of pharmacophores, chemical building blocks that had worked for him in the past. From then on, chemically speaking, 'one thing led to another' and between 1956 and 1984 he invented and brought to market 35 new drugs. No one else has ever achieved such success (Lewi, 2007).

These three great men, Ehrlich, Domagk and Janssen shared a number of approaches to drug discovery. They started from molecules that they already knew had selective affinities for tissue components, they tested new compounds in whole animals or, in Janssen's case, additionally in isolated-tissue bioassays and they used the pharmacological results of these tests to synthesize new molecules based on informed ideas about structure-activity relations. Thus, they all used the iterative, evolutionary, selectionist approach that Charles Darwin had recognized as the basis of successful biological speciation. Although each of them had an initial conception, for their conception to lead to a successful invention based on that conception three other psychological components were necessary. These were concentration – they allowed nothing

and no one to deflect them from their enterprise; commitment – they stayed with their project as long as was needed to complete their task and, finally, creativity – not just the final patenting of a molecule but the act of deciding that a molecule was 'fit for purpose'. I believe that concentration and commitment have got more to do with character than with intellect.

All successful new drug research programmes begin with a molecular starting point which is already known to have tissue selectivity. Ehrlich and Domagk started from dyestuffs, tissue-selective by definition; Janssen started from pethidine, a molecule already known as an effective drug due to its tissue-selectivity for nervous tissue; and, in 1958, I started from the knowledge of the tissue-selectivity of the drug, isoprenaline, which was being used clinically at that time for the relief of asthma.

My interest in isoprenaline arose from an idea I got for treating both angina pectoris and the sudden death associated with that syndrome due to the precipitation of ventricular fibrillation. Angina was well known to be the result of the coronary arteries becoming narrowed by atheromatous disease. So, in the early 1950s, all the research efforts were being directed to trying to increase the coronary blood flow. After all, for years patients had been taking nitroglycerine tablets for anginal pain. With the onset of an attack, patients would stop, fumble with their pill box and put a tablet under the tongue. Patients were aware that rapid relief was associated with a warm facial flush. It was generally assumed that a similar vasodilatation was taking place in the coronary blood vessels bringing relief whereas the mere of stopping to find their pill box may have been enough! Nevertheless, industrial pharmacologists invented new drugs that were selective coronary vasodilators in healthy anaesthetized dogs; but laboratory success invariably ended up in clinical failure. The Baltimore surgeon, Claude Beck, tried to increase the collateral circulation to the heart by stitching the omentum to the scarified surface of the pericardium; again surgical success ended up in therapeutic failure. From 1950, I was teaching mammalian physiology to veterinary students and running my own research lab. Mr George Smith, a young cardiac surgeon, who had trained with Beck joined me in my lab to develop an idea that he had got while he was working with Beck. His idea was to increase the amount of oxygen being carried by the blood. He showed that when a major coronary artery in the hearts of anaesthetized dogs is tied, nine-tenths of animals developed ventricular fibrillation within 2 h. However, when the dogs were put in a high pressure chamber filled with 100% oxygen at 2 atmospheric pressure, then only one-tenth of dogs fibrillated in the same time period. Of course I knew that haemoglobin was fully saturated with oxygen at the normal atmospheric pressure of a fifth of an atmosphere. Therefore, the protection was being given purely by the oxygen dissolved in the blood plasma – and oxygen has a very low solubility in water. I calculated that the oxygen content in blood exposed to two atmospheres oxygen pressure increased by only 10–15%.

My idea was that if such a small increase in oxygen supply was so effective, then, perhaps, an equally small decrease in the heart muscle's demand for oxygen would also be effective. The main determinant of oxygen demand is heart rate. The main determinant of heart rate is the frequency of nerve impulses in the sympathetic nerves to the heart. The sympathetic nervous system gears up all our organs to deal with the physiological emergencies of flight, fight and fright. It was well known at the time that the sympathetic nerves instructed heart muscle and other responding cells by the secretion of adrenaline (noradrenaline was still to be discovered). Therefore, I wanted to find a drug that would block the effects of adrenaline on the heart. However, the sympathetic hormone, or messenger molecule, is delivered to every organ in the body. Consequently, a generalized blockade of all of the actions of adrenaline would surely also have the undesirable properties of reducing our physiological capacity to deal with emergencies. In particular, the sympathetic nervous system maintains and adjusts blood pressure by variable constriction of all the small blood vessels in the body. Without the reactivity of the sympathetic nervous system, every time we stood up we would faint. We knew this was true because of the effects on blood pressure of the anti-adrenaline drugs that had been invented by Fourneau in the late 1920s. These drugs had no effect on blood pressure when the subjects were lying down but when they stood up their blood pressure fell and they became weak and dizzy. When blood pressure falls, the blood flow through the coronary arteries decreases and so must increase the risk of heart attacks. So, my idea of adrenaline blockade to relieve angina almost died at its birth – it could have had the opposite effect in that any benefit from reduction in heart rate would be offset by a reduction in blood flow through the coronary arteries.

Although I was teaching mammalian physiology to veterinary students at that time, in 1954 I bought the first edition of a new multi-author textbook on 'Pharmacology in Medicine' edited by Drill (Levy and Ahlquist, 1954). To my excitement, I found a chapter on Adrenergic Drugs by Ray Ahlquist (1948). He was able to use this opportunity to expound his own highly original ideas. He had had

great trouble getting his original work and ideas accepted but his paper was eventually published in1948 in the American Journal of Physiology largely because the Editor, WF Hamilton, was a colleague and personal friend. Nevertheless, for the next 10 years or more his work was completely ignored. So it was very unusual, but lucky for me, that such a controversial pharmacologist had been given so much space in a medical textbook. Ahlquist had been struggling to understand the pharmacological properties of isoprenaline. Isoprenaline, a very simple derivative of adrenaline, had retained adrenaline's ability to excite or stimulate the heart and inhibit or dilate the bronchi but had lost adrenaline's ability to excite or constrict blood vessels. So he had proposed that there were two kinds of adrenotropic receptors which he labelled alpha and beta. It was this concept that hormones and other messenger molecules achieve their effects by acting on receptors on the surface of the responding cells that was revolutionary and that physiologists found so hard to swallow. He proposed that when adrenaline was stimulating blood vessels to constrict and raise blood pressure, it was activating alpha receptors, but when it was activating receptors to speed up the heart and dilate the bronchi, it was acting on beta receptors. He proposed that Fourneau's anti-adrenaline drugs were selective antagonists of alpha receptors and so led to the fall in blood pressure on standing. Both doctors and patients had known that the dizziness on standing was accompanied by a palpable increase in pulse rate, a beta-adrenotropic receptor mediated effect. In his model, isoprenaline was a selective 'agonist' at beta-adrenotropic receptors which explained its clinical use for treating asthma by dilating the bronchi, a useful effect which was restricted by inducing tachycardia. On the other hand, Fourneau's anti-adrenaline drugs were selective 'antagonists' at adrenaline's alpha-adrenotropic receptors hence leading to a fall in blood pressure on standing. This was the first time that the receptor idea had been used to explain the differing properties of hormones and drugs related to them.

It was now clear to me that what I wanted was a selective 'antagonist' active only at the beta receptors. Remember that in the 1950s 'receptors' were mere concepts. Ahlquist, in his autobiography (Ahlquist, 1973), describes how he thought that his alpha and beta receptors were not like pebbles on a beach, that their existence lay inside his own head, that in fact he had made an invention whereas we now know that he had made a discovery that they do indeed exist as gene products.

All new drug research programmes have to begin with a chemical starting point. Ehrlich and Domagk started from dyestuffs, Janssen started from pethidine and, in 1958, I started from the knowledge of the tissue-selectivity of the drug, isoprenaline, which was being used at that time by inhalation to relieve asthma by relaxing bronchial muscle and dilating the airways. My idea was based on the concept of hormones and their corresponding receptors.

I broadly define receptors as 'any devices that receive information, signals, etc.', a concept that is now widely used in biology. Physiologists refer to sensory receptors, chemoreceptors and baroreceptors while microbiologists talk about viral receptors and, ever since Clark in 1926 (Clark, 1926) pharmacologists have talked about drug receptors. Thus 'receptor' only has meaning when it carries a prefix. The problem with Clark's prefix of 'drug' receptors is that it does not carry any physiological implication. I have long suggested that pharmacologists should do the same and use the prefix 'hormone' to the receptors that interest them. Bayliss and Starling in 1902 introduced the idea of a hormone, a word derived from the Greek word for messenger (Bayliss and Starling, 1902). They observed that when food enters the upper part of the intestine, the chemistry of the food stimulates the secretion of a messenger molecule into the blood stream by cells that line the lumen of the duodenum. They called their new hormone 'secretin' because it stimulated the pancreas to secrete digestive enzymes into the duodenum. Thus they had invented a new, generic, word for any messenger molecule – a brand new idea in physiology in 1902. Prior to this time, physiologists had believed in Pavlov's Doctrine of Nervism – that the functions of all our internal organs were regulated by nerve reflexes Since the discovery of secretin, physiologists have found many messenger molecules that are secreted into the blood and so are distributed to every cell in the body. Whether or not a cell responds to the messenger is determined by whether or not that cell expresses corresponding receptors on their surfaces. Most of the circulating hormones come from the endocrine glands such as the pituitary, thyroid and ovary and the corresponding hormones are growth hormone, thyroid hormone and oestrogens.

Unfortunately, pharmacologists, thinking to pay homage to Bayliss and Starling, have worked on the basis that if a messenger molecule is not circulated it is not a hormone. Unfortunate because we now know that all of the 200 or so different kinds of cells that make up our bodies 'talk' to each other by sending out chemical messages. 'Unfortunate', because we now recognize many different kinds of messenger molecules but refer to them variously as neurotransmitters, chemicals that are secreted at

nerve endings; paracrines, chemicals that are used in a conversation between adjacent cells; and autocrines, chemicals that a cell makes to talk to itself. There are thousands of these messenger molecules with their corresponding receptors. Logically, I believe, l we should lump them all under one class, that of hormones with the corresponding class of hormone receptors. But biological scientists are not always logical, especially when hallowed traditions are at stake.

During the last 100 years or so, the evolution of our ideas about hormones and their corresponding receptors has been increasing exponentially. Fifty years ago, endocrinologists talked about hormones but never felt the need to talk about their cellular receptors. The only people who talked about receptors were a small group of mathematically minded pharmacologists who tried to understand the quantitative relations between the size of the concentration of the hormone and size of the corresponding tissue response. For their equations, they needed the molecules of the hormone to interact, on a one-to-one basis, with unspecified, and, at that time, unspecifiable, tissue molecules or receptors. The pharmacologists of my youth were embarrassed by the molecular vagueness of their idea. In my 50 years in research, I have seen the pharmacological concept of a receptor change in five definable steps.

1. An abstract chemical concept.
2. An explanatory model, first used by Ahlquist to explain the selective tissue effects of isoprenaline on notional alpha and beta receptors.
3. A trigger for the production of specific intracellular second messenger molecules (Haynes *et al.*, 1960).
4. The contributions from Changeux (Heidman and Changeux, 1978) and Lefkowitz (Heinsimer and Lefkowitz, 1982) who independently showed that receptors could be extracted from tissues as unique chemical entities at can be switched on and off as physiologically appropriate or as pathologically determined (Tanaka *et al.*, 2003).
5. Gene products capable of polymorphic expression.

Overall, this exercise has, so far, taken about 60 years, in effect my lifetime in and around pharmacology labs and, I predict it will still be running 50 years from now. In passing, note the slow rhythm of science that cannot be hustled by government, or venture capitalists or granting bodies. As Thomas Kuhn argued 40 years ago (Kuhn, 1970), progress in science is limited by the initiation of new concepts and not by the accumulation of information. Data and results are additive but concepts and ideas are substitutive. A new concept has to displace an older one and shedding time-honoured concepts can be difficult, even painful, hence the friction that delays progress.

A hormone must first bind selectively to its own specific receptor, known as the hormone's affinity, and then the hormone must instruct or trigger the responding cell to change its behaviour, the property known as efficacy. In the 1950s, Stephenson (1956) and Ariens (1954) showed independently that, in a series of hormone analogues or derivatives, affinity and efficacy were separable properties. Synthetic hormone analogues, now known generically as agonists, could be fully effective, or lose some efficacy without loss of affinity therefore known as partial agonists, or lose efficacy altogether while retaining binding affinity for the receptor. These latter compounds could now compete with the corresponding agonist for receptor occupation and so would annul the agonist's effectiveness, hence their description as competitive antagonists. In this receptor model, affinity refers to the fraction of receptors occupied at equilibrium and hence its measurement is independent of receptor density and its value can be measures on any tissue. Efficacy, however, is a multiple of occupied receptors and so its value will vary from tissue to tissue. Hence, a 'partial' agonist can behave like a full agonist on a tissue with high receptor density and a competitive antagonist on another tissue with low receptor density. On my first project, before I understood this theory, I was often confused by my results.

Specifically, by 1956, I wanted to find a competitive antagonist for adrenaline at its beta receptors. The structure of isoprenaline seemed to point to how I might invent a competitive antagonist in theory but reduction to practice required funding. I had got to know the Imperial Chemical Industries (ICI) Pharmaceuticals representative in Glasgow and through him I was site visited in my Glasgow lab by senior members of their Research Division. They invited me to join ICI in their new, state-of-the-art, labs in Cheshire to tackle my project. I accepted and moved in June 1958. A new colleague the chemist John Stephenson joined me to work on the project.

The easy part of our problem was to measure the pharmacological effects of isoprenaline. For this, I was able to use the Langendorff preparation, the isolated perfused guinea pig heart much used in student practical classes. The hard part was to choose the chemical starting point for our chemical programme. Adrenaline is derived from phenylalanine, an essential amino acid that is one of the building blocks of proteins and the physiological source of a simple molecule known as phenylethylamine. It is an asymmetrical molecule with a

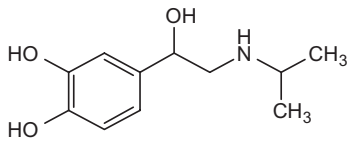

Figure 1

The chemical structure of isoprenaline – the starting point of the quest for a beta blocker.

benzene ring attached at one end of a 2-carbon chain and a basic nitrogen atom at the other end. Adrenaline has two reactive hydroxyl groups attached to the benzene ring and has a methyl group attached to the nitrogen atom. Isoprenaline is a close chemical relative of adrenaline having an isopropyl group instead of the methyl group on the nitrogen atom – hence the name 'isoprenaline' from isopropyl-adrenaline.

Isoprenaline neither stimulated alpha receptors, nor bound to them, so it did not block the effects of adrenaline at alpha receptors that would have resulted from the competition for binding between the active and inactive molecules. I could have deduced from this that if the selective binding to the receptors was a function of the nitrogen end of the molecule, then, perhaps, efficacy would be associated with the other end of the molecule, the substituted benzene ring – but I did not! We stayed with the nitrogen end of the molecule and tried replacing the isopropyl group with larger and larger phenylethyl and related groups (Figure 1).

Then, early in 1959, we came across a paper that described the properties of the dichloro-benzene analogue of isoprenaline, known as DCI. Powell and Slater, the inventors, were trying to make a long-acting bronchodilator by replacing the readily metabolisable ring hydroxyl groups of isoprenaline with metabolically stable chlorine atoms. They did not succeed because DCI had lost all its agonist activity at bronchial muscle. In their paper they reported that after the bronchial muscle had been incubated with DCI, isoprenaline seemed to lose its agonist activity but they drew no conclusions about the possible significance of the phenomenon (Powell and Slater, 1958). Stephenson quickly synthesized DCI for me and we found that it was as potent an agonist as isoprenaline on my cardiac pacemaker (Langendorff) preparation. As we had no idea what this meant, the compound was temporarily shelved.

Meanwhile, I was busy trying to develop a new bioassay. The primitive 'smoked-drum' recording used with the Langendorff preparation meant that the amplitude of the recording was a compound of changes in rate and force of contraction. However, a recently described assay measured the force of contractions of electrically stimulated (fixed-rate) papillary muscles from the right ventricles of kitten hearts. I have no strong anti-vivisection views, but drew a line at killing kittens although from a practical point of view, kitten muscles were ideally relatively long and thin. My chosen alternative, guinea pig muscles, were short and fat. Nevertheless, I was able to develop a satisfactory assay from guinea hearts. When I retested some of our old compounds, I retested DCI. On this new preparation DCI did not augment the force of contractions but now antagonized the increments produced by adrenaline or isoprenaline. I vividly remember John Stephenson rushing into the labs the next morning. He explained the electronic differences between a chlorine substitution and a hydroxyl group. However, his really exciting news was that the two chlorine atoms on the phenyl ring had about the same special occupation as a naphthyl ring but without the electron-withdrawing effect of the chloride substituents. We found on the two bioassays that this new compound blocked the effects of isoprenaline on the papillary muscle preparation but did not augment its effects on the Langendorff preparation.

However, another two years, and many new syntheses, were needed before our invention of propranolol, the first beta blocker to reach the market under the name of Inderal (Black *et al.*, 1964). Beta blockers did indeed give relief to patients with angina but not as much as the coronary by-pass operations that were subsequently developed. Another discovery made in the clinic, was that beta blockers were very effective at reducing the blood pressure of patients with hypertension and unlike other antihypertensive drugs available at that time, they did not make people feel dizzy. The main side effect was that some patients did develop Raynaud's phenomenon. In addition, the benefits of beta blockers in treating cardiac arrhythmias, angina and hypertension, we have seen in the last decade a remarkable increase in their use for treating chronic heart failure.

Thus, my first contribution to new drug research began empirically. It grew into an exercise in lateral thinking bringing together ideas about hormones as chemical messenger molecules and their corresponding cellular receptors. The chemical part of the programme began with the known structure of isoprenaline, a close relative of adrenaline the natural hormone. The bioassays were based directly on the objective of inhibiting the cardiac responses to adrenaline. I cannot be certain that I would have seen the possibility of attacking other projects where inhibiting the actions of other physiological messenger molecules could be seen to have possible

value in related disease processes but by accident such a project was at hand.

Part 2

Rational approaches

My work on beta blockers had begun in my labs in the Veterinary School in Glasgow where I had the privilege of collaborating with George Smith. George Smith had a colleague, Adam Smith, a Gastrointestinal surgeon who had just returned from a sabbatical year at the National Institute for Medical Research where he was studying 5-hydroxytryptamine (5-HT). He noted that 5-HT seemed to increase the secretion of gastric mucus. He wondered if it might inhibit histamine-stimulated acid secretion. In the event, we found that 5-HT did indeed inhibit histamine-stimulated gastric acid secretion and increased the secretion of mucus. Through Adam Smith, I heard about his colleague, Andrew Kay's work on the development of the 'Augmented Histamine Test' that he was developing to estimate the size of the parietal cell mass to help assess the role of gastric surgery in the treatment of peptic ulcers. In this test he used large doses of the antihistamine mepyramine to suppress the very unpleasant side effects of parenteral histamine. Looking back, I am astonished that I cannot recall anyone including me expressing surprise that the stimulation of acid secretion by parenteral histamine was not blocked by the antihistamine drug. I remembered this work as my beta-blocker programme was reaching the clinic. Having achieved the 'beta blocker' target, I began to wonder if histamine too might have its beta receptors.

When my beta-blocker programme was coming to an end in 1964, there was little interest in histamine among physiologists interested in how food makes the stomach secrete acid because of the excitement about gastrin. Remember my earlier comments about 1902 and the discovery of the hormone secretin. In 1905, Edkins thought he had found a similar hormone, released by food, which was secreted into the blood by a part of the stomach called the antrum (Edkins, 1906). He was a good experimenter and showed that the antral extract stimulated acid secretion and lowered blood pressure. However, an extract of the acid secreting region of the stomach also lowered blood pressure but did not stimulate acid secretion. He called the new hormone 'gastric-secretin' or gastrin for short. However, it was soon shown that extracts of almost any tissue would lower blood pressure. Then these extracts were shown to contain histamine and, finally, that histamine could stimulate acid secretion. There was plenty of histamine in the stomach wall so, somehow or other, histamine secreted locally could be the agent. So, Edkins' 'gastrin' hypothesis seemed to have failed. Indeed, in 1926, Ivy concluded that 'gastrin is histamine' (Sacks *et al.*, 1932).

Then, in 1938, there was an interesting coincidence. Boris Babkin, who had been a pupil of the great Pavlov in Moscow, was now Professor of Physiology at McGill University in Montreal. Babkin had been indoctrinated with Pavlov's concept of Nervism – that the function of all organs was controlled by nerves. However, Babkin had become convinced that secretin was a hormone that controlled pancreatic secretion and was dubious about the claims that gastrin and histamine were identical – so, he encouraged his staff and research students to work on the problem. In 1938, McIntosh came from Oxford, where he had done his doctoral work with William Paton working on the new 'histamine-releasing agents', to be a postdoc with Babkin. McIntosh showed that stimulation of the nerves to the stomach released histamine in its acid-secreting region (MacIntosh, 1938). Komarov was working in a lab next door. He was a biochemist who decided that if gastrin was a small protein and not a small base like histamine then a different method of extraction was needed. He showed that there was, indeed, a hormone gastrin secreted by the stomach that was not histamine (Komarov, 1938). Rod Gregory, who had done post-graduate studies with AC Ivy in Chicago during the war, took up the problem when he returned to Liverpool. By 1964, he had extracted pure gastrin from stomach tissues of abattoir pigs. He showed that gastrin was a small, 17 amino acid protein. The peptide was then synthesized by his colleague George Kenner. The consensus view was that acid secretion had its own hormone and so there was now no need for involving histamine in the physiological control of acid secretion (Gregory and Tracy, 1964).

However, there was a lonely voice in opposition, that of Georg Kahlson from Lund in Sweden. He was a biochemist interested in the enzyme, histidine carboxylase, which makes histamine from the amino acid histidine. He was showing fairly conclusively that, in the stomach, gastrin activated that enzyme and increased the formation and secretion of histamine in the stomach wall (Kahlson, 1960). So, if gastrin stimulates the stomach to secrete acid and if it does this indirectly by releasing histamine, why do the antihistamine drugs not block acid secretion?

My working hypothesis was that there were alpha and beta histamine receptors that would correspond to the alpha and beta adrenaline receptors.

If so, then histamine acted on its alpha receptors to contract muscles of bronchi and intestine and Bovet's antihistamines would be alpha receptor antagonists. In addition, histamine might be acting on its beta receptors to stimulate acid secretion. Editorial pressure later forced me to refer to these histamine-related alpha and beta receptors as histamine H1- and H2-receptors. I reasoned that if I could find a histamine H2-receptor antagonist I would be able to resolve the hen and egg argument between histamine and gastrin, and, if Kahlson and McIntosh were right, I might have found a way of inhibiting the over-secretion of acid that was the problem in gastric and duodenal ulcer disease. This was what is nowadays referred to as blue-sky thinking with a project of uncertain duration and very uncertain outcome. To obtain the necessary research funding, I had to move to another pharmaceutical company.

New drug invention is a team exercise and in 1964 my chemists started their synthetic programme with histamine, which is like adrenaline, derived physiologically from an essential amino acid. Like adrenaline, histamine is a small asymmetric molecule with a 5-membered ring of atoms, known as an imidazole ring, at one end and a 2-carbon atom chain ending in a nitrogen atom at the other end. We started by chemically modifying the ring end of histamine. Our initial bioassay was the lumen-perfused isolated guinea pig stomach that was driven to secrete acid by histamine in the perfusion fluid. Very soon we found a simple modification that turned histamine into a selective H2-receptor agonist, the equivalent of isoprenaline at beta receptors. The compound, ring-substituted 4-methylhistamine, stimulated acid secretion without contracting the muscles of bronchi and intestine. This was my expectation for the behaviour of a selective histamine H2-receptor agonist. I knew that I was on the right track. This discovery was to be the thin gruel that kept me going for the next four years. Remember that isoprenaline became a selective agonist by modifying the end of adrenaline with the nitrogen atom and the antagonists came from modifying the end with the benzene ring. The selective histamine agonist, with its the loss of affinity for the histamine H1-receptor, came from modifying the ring end of histamine so, by analogy, it was the side chain end with the nitrogen that must be conferring agonist efficacy. So, from a chemical point of view, I should have immediately attacked the nitrogen, but I did not. It took 3 years of slow and difficult chemistry before I realized my error.

Eventually, we found that replacing the amino group with a guanidine group produced a partial agonist that was a histamine-like agonist on acid secretion but a weak antagonist to histamine on uterine muscle. The guanidine analogue of histamine was to H2-receptors what DCI was to beta receptors. Lengthening the chain of atoms that joined the guanidine group to the imidazole ring produced more potent compounds. My group led the subsequent chemical evolution that resulted in cimetidine (Figure 2) which was eventually marketed as Tagamet® (Brimblecombe *et al.*, 1975).

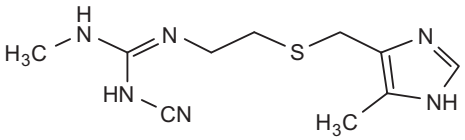

Figure 2
The chemical structure of cimetidine (Tagamet®).

This project took about 9 years from start to finish and the drug was useful because Kahlson was right – gastrin does indeed stimulate acid secretion indirectly by releasing histamine. Gastrin stimulates its own receptors on the enterochromaffin-like, or ECL, cells to release the histamine that stimulates the H2-receptors located the acid secreting cells adjacent to each other in the mucous membrane of the stomach. Histamine H2-receptor antagonists turned out to be able to heal ulcers of stomach and duodenum, thus greatly reducing the workload of gastro-intestinal surgeons. Patients who had previously had to live with significant morbidity following complex gastric surgery now only had to take oral medication.

However, there was a problem. The clinical experience with cimetidine was that after the patients' ulcers had healed in 4–6 weeks, many of them rapidly relapsed. Everyone assumed that a patient's tendency to produce ulcers in the first place, was still present and produced the relapse. However, gastroenterologists had gone on to show that H2-receptor blockade raised the blood levels of gastrin. By this time, Johnson (Johnson *et al.*, 1969) had discovered that gastrin not only stimulates the ECL cells to secrete histamine but it also stimulates them to grow and divide, a condition known as hyperplasia. Consequently, when the H2-receptor blockade was withdrawn, gastrin now released more histamine than ever from the ECL cell hyperplasia and hence there was a much greater secretion of acid. Could this be involved in patients' relapse? A simple way to find out would be to invent a gastrin receptor antagonist.

Gastrin is a polypeptide meaning that it is composed of a chain of 17 amino acids. However, all of the agonist activity of gastrin is given by the

terminal 4 amino acids known as tetragastrin. We made a breakthrough straightaway when we showed that this small molecule ties itself into a tight spiral or helix (Black and Kalindjian, 2002). So we developed some beautiful *in vitro* assays and, in the usual way, set about trying to make analogues that had lost efficacy but retained affinity. Over the next 10 years, we made many gastrin antagonists that worked beautifully in the *in vitro* bioassays but which regularly failed to be usable drugs when they were tested in animals due to properties such as poor absorption from the stomach, or extremely rapid elimination by the liver. However, after completing three small clinical studies we have grounds for optimism that we will win in the end.

In the last few years, there has been growing unease among gastroenterologists that drug-induced increases in the blood levels of gastrin may be associated with the worrying increases in the incidence of cancer of the oesophagus, stomach and pancreas. The new highly potent acid-secretion inhibitors, the proton pump inhibitors, are particularly suspect by some gastroenterologists. These are problems that a new gastrin receptor antagonist might help to illuminate. We have shown in a small, placebo-controlled study, that one of our gastrin antagonists, free of side effects, but which has to be delivered by continuous intravenous infusion can more than double the median survival of patients with pancreatic cancer (Black, 2009). We now know that pancreatic cancer cells develop gastrin receptors and like ECL cells respond to exposure to gastrin by an increase in their rate of growth. So, every time patients with pancreatic cancer eat they stimulate their rate of tumour growth. Perhaps this is why pancreatic cancer is known as 'the dismal disease'.

Note the long time scale that can be involved in new drug invention – as we have been working on the gastrin project since 1989.

In each of these three projects, I have asked my colleagues in medicinal chemistry to emasculate a hormone and invent its corresponding antagonist. This conception, the invention of selective hormone receptor antagonists has been used successfully by many research groups. Indeed, the invention of many different hormone-specific antagonists has been one of the great success stories of the pharmaceutical industry. This is the background to my belief that it is possible to generalize a strategy for drug research. A drug research strategy is the set of principles that help one to decide what to do in the lab with the clinic in mind. My principles are based on my own experience and reading about the work of others. They start with a desire to develop a new drug with a new pattern of selective effects that, if they occurred in man, would have therapeutic merit. My seven principles are:

1. I must have evidence, before I start, that the desired selectivity is capable of being expressed by a drug. There may be empirical evidence that other drugs are already known to possess that selectivity and then there is no issue to settle. However, when the desired selectivity is new, then, unless there is evidence of the biochemical differentiation that will allow the selectivity to be expressed, the project is likely to be based on mere wishful thinking and probably doomed from the start.
2. There must be an explicit chemical starting place. Medicinal chemists must have a molecular template with the command 'Begin here'. The chemical template may be an already known drug or it may be a natural, physiologically important, molecule or even, today, a lead generated by random screening – but they have to start somewhere.
3. There has to be a biological measurement, or bioassay, which can measure the specific property imagined to underlie the intended selectivity – in my experience, a functional bioassay is always preferable to a surrogate marker.
4. Before I start, I have to be able to envisage how I will be able to show in man that the drug has the same basic actions as I would have found in the laboratory.
5. I have to have a disease in mind in which it would be logical to evaluate the new drug's properties.
6. I or one of my colleagues must feel passionate about the project – drug research programmes can go through long periods, as much as 4–5 years, when little progress seems to be happening – then passionate conviction is what keeps you going.
7. Finally, there must be a reasonable likelihood of adequate funding to complete the project.

If the project that is picked is to try to invent a new hormone receptor antagonist, the inventive process works in the following manner. Once the chemical structure of a hormone is known, the medicinal chemist can, in his imagination walk around the molecule and try to guess the chemical features that make it efficacious. An initial guess can be evaluated by making an appropriate analogue or derivative of the hormone. The intuition and experience of medicinal chemists still seems much more reliable than molecular modelling. The new compound has to be evaluated by bioassay, a piece of tissue or tissue culture that responds characteristically to the

hormone. The new compound can be tested alone – is it still efficacious? – and then in combination with the hormone – does it antagonize the hormone? At this stage negative answers are as informative as positive ones. Either way, the results suggest new chemical questions, new compounds to be made and tested. This basic iterative loop – synthesize, test, synthesize – has been shown to work reliably. With luck, compounds with a partial loss of efficacy are followed by weak antagonists leading eventually to very potent antagonists.

So far, I have been describing the search for new drugs in the pharmaceutical industry as I have known it for 50 years. However, within the last 25 years or so, an extraordinary revolution has been taking place within industrial pharmaceutical research companies. The desire for medicines in the hope of achieving relief from discomfort is huge. People will spend money on over-the-counter medicines in preference to many other necessities of life and in a world with increased longevity and a higher proportion of elderly citizens the demand for drugs aimed at alleviating chronic disease and degenerative conditions will continue to rise. The downside of the market is that the monopoly phase of patent-protected marketing is being progressively eroded as obligatory development times have increased. The costs of research and development have been soaring, partly because of regulatory requirements for extended Phase 3 trials, and partly by the rising costs of the new technologies. On the other hand, there has been huge pressure from governments and health maintenance organizations to reduce the prices even of monopoly drugs. In-house research and development productivity had to increase. Today, the founders of the craft of drug invention, such as Ehrlich and Janssen, are seen as slow-footed 'dinosaurs', their approach superseded by the asteroid of rapidly moving new technologies.

These new technologies are based on advances in genetics and molecular biology. Hormone receptors, for example, can now be extracted from cells as gene products and expressed on the surfaces of tiny glass or polymer beads or on naïve, specific-receptor free cells. Reporter systems expressed along with the receptors 'light up' when a drug molecule binds to the receptor. Reactions between drug and receptor molecules take place in a thousand tiny wells in a Perspex plate. In this way, robots can dispense and screen many thousands of molecules in a day. This is known as High Throughput Screening or HTS. Another revolution, known as combinatorial chemistry, or Combichem, has developed concurrently. These chemical techniques were developed to satisfy the screening appetites of HTS. It is important to understand that Combichem only works with simple reactions that generate high yields, sometimes referred to as pre-ordained chemistry. Many of the molecules that were made by the older iterative strategy could not have been produced by Combichem. In-house Combichem has now been replaced by purchasing huge lists of chemicals from suppliers of chemicals of no particular origin. New drug research used to be concept driven, now it is technology driven. However, the industry-wide deployment of the new technologies is now seen to be associated with a worrying decline in real, innovative, productivity. The reason for the decline now being given is that the successful 'dinosaurs' solved the simpler problems, sometimes described as clearing out 'the low hanging fruit'. There may be some truth in that claim but I want to offer other possible explanations.

First, take the strategy of high-throughput screening of huge, small molecule, databases. The first assumption is that these blindly synthesized libraries are a reliable source of new leads. As a lead generator, Combichem plus HTS undoubtedly works and complexity specialists, such as Gerald Kaufman, argue that life began as an exercise in combinatorial chemistry. Primitive bacteria-like and tube-like forms seem to have been around for over 2.5 billion years before the Pre-Cambrian explosion started structural evolution 500 million years ago. Maybe natural selection had first to operate at the level of combinatorial chemistry, chemical evolution if you like, to develop huge populations of molecules that were comfortable with each other before a stable basis for structural evolution could take off. My reading of the history of drug inventions suggests that the most selective drugs, with the widest therapeutic ratio, have come when the initial lead was a native, physiological, molecule. Perhaps drugs that are crafted round a natural template retain some of the parental selectivity. So my first caveat, to the Combichem plus HTS strategy, is that all leads may not have the same quality. Remember that while the new tactics generate leads, the older strategies never started without leads and they still took years to exploit. As to the effectiveness of the new tactics, there are already worrying signs that the increasing numbers of compounds going into early clinical trials are failing to reverse the declining numbers of compounds that reach the phase of registration. Is this due to inadequate selectivity that only becomes visible when amplified by the large patient numbers used in Phase 3 studies?

Now, consider how we have arrived at our present situation. Our knowledge of genes, and the proteins that they specify, plus all the complexities of molecular biology have been achieved by the scientific process of reductionism. Reductionism has

proven to be our most successful analytical tool. Anatomists and physiologists have progressively deconstructed the form and function of whole persons into their organs and tissues and then into the various cells that make up these tissues. Biochemists and molecular biologists have subsequently reduced these cells into the huge number of molecular components that are the subject of modern biochemistry. Organisms, tissues and cells are certainly composed of these molecular components. However, as they interact with each other they form a system that, like the psychoanalyst's idea of gestalt, is more than the sum of its parts. Components are to systems as words are to poems and pigments are to paintings. The decomposition of poems and paintings into words and pigments is not reversible. My concern about the current thrust of drug research is that it is rooted on targeting components rather than systems. I think that this becomes apparent when we consider the new drugs that the pharmaceutical industry is seeking, to treat disorders such as asthma, dementia and cancer, are expected to be similar to the ones that they have already invented to reduce high blood pressure, heal stomach ulcers and relieve pain. I am concerned that this expectation may not be fulfilled. As I see it, the problem has to do with the difference between molecular components and physiological systems. Physiology is about how cells use chemicals to talk to each other. Sometimes, the message has the shape of a command, such as 'contract' or 'secrete' or 'move'! Thus, adrenaline is the final messenger to the pacemaker of the heart in emergency situations. So a drug that blocks the effects of adrenaline on the heart effectively controls cardiac stress responses. Note that the heart must react to stress reliably, on cue, but if it beats faster inappropriately nothing very bad happens. The process of heart rate changes is inherently reversible. Some of our most useful drugs act by interfering with chemical commands. Cells that respond to commands are behaving like analogue devices. As analogue devices, these cellular processes are inherently unstable. Just as an equestrian controls a thoroughbred horse in traffic by simultaneously urging the horse forward with his knees and restraining it with his hands through the reins – control being achieved by the balance between stimulation and inhibition – so cells with analogue behaviour are being stimulated and inhibited simultaneously, the yin-yang of physiology. The cardiac pacemaker cells are simultaneously being driven by nor-adrenaline released from the sympathetic nerve endings and inhibited by acetyl-choline released from the parasympathetic nerve endings. Acid secreting cells in the stomach are simultaneously being stimulated indirectly by gastrin and inhibited by somatostatin. As far as I can see, this is a fundamental design principle that applies to every cell that exhibits reversible, analogue behaviour. The physiological problem that has barely been addressed is how the balance between driving and braking is sensed at the regulatory level. At a higher level, each control arm is subject to some kind of feedback control about which our reductionist efforts have left us even more ignorant. So we can become frustrated by our pharmacological predictions.

However, there is another kind of physiological system that must also be activated reliably but which, if activated inappropriately can have damaging, even lethal, effects. Examples of these systems are commitment of stem cells, activation of killer lymphocytes, cell division and growing new blood capillaries. Once initiated, these are inherently irreversible processes. These cellular responses are like switches, on-and-off digital devices. So, how are these physiological processes controlled such that they can be activated on cue but never inappropriately? The striking feature of these irreversible processes is that many chemical messengers are involved, each having a different cellular origin. A feature of these messenger molecules is that they can often be shown to potentiate each other. So I imagine a process that I call 'convergent control'. I imagine that an effective stimulus might involve the co-operative interaction of more than one agent involving addition or amplification of, individually, subliminal stimuli. I imagine a growth factor giving a stem cell, say, not a command, but a piece of advice, such as 'Other things being equal, you should start dividing'! The other equal things are other chemical messengers, which have to impinge on the cell at the same time to achieve its activation. This advise-consent arrangement leads to information-rich management. Physiological control by chemical convergence entails the possibility of redundancy. Therefore, annulling the action of a single component may be disappointing. Biotechnology has been hugely successful at blocking the actions, individually, of various molecules known to be over-produced in septic shock. However, in every case, laboratory success ended up in clinical failure. At some point we must ask whether the model or our way of thinking is wrong. If there is any truth in this idea, the conclusion is plain. Physiological systems that are organized by chemical convergence based on potentiating interactions will need pharmacological convergence to manage them effectively. There is now a vast literature describing potentiating interactions between intercellular messenger molecules. However, I am not aware that anyone has proposed a Theory on

Potentiating Interactions at the molecular level. We need to develop a conceptual base to allow us to predict the best pharmacological combination.

In this essay, I have not tried to hide my prejudice for having *in vitro* bioassays at the heart of any new drug research programme. I want to end by describing how the discovery of nitric oxide as a major chemical messenger could not have been made any other way. Here is the story. When acetylcholine is infused intra-arterially in the forearm in man, the blood vessels dilate. Furchgott studied the effects of drugs on rabbit arterial muscle isolated in organ baths. At one time, the only way to measure the effects of drugs on arterial muscle was by measuring changes in its length. To get measurable shortening, the blood vessel was cut into a long spiral strip. In this preparation, Furchgott found that acetylcholine had no relaxant effects, indeed the muscle usually contracted. When instruments for measuring tension became available, isometric measurements could be made on rings of arterial muscle where significant shortening was no longer needed (Furchgott and Zawadski, 1980). Imagine his surprise when Furchgott found that acetylcholine now had the expected relaxant effects on arterial muscle. He went on to show that the endothelium was intact in the muscle ring but was destroyed in cutting the muscle spiral or by rubbing the lumen of a muscle ring. He showed that acetylcholine relaxes arterial muscle indirectly by stimulating the endothelium to secrete a relaxing factor that diffused into the adjacent muscle layer. This seminal discovery was only possible when the muscle and endothelium were in direct physical contact combined, as it were in a system. We now know that the relaxing factor is a gas, nitric oxide, which escapes when endothelial cells are cultured *in vitro*. So this astonishing discovery could not have been made by the powerful, component-directed techniques of molecular and cellular biology. Whatever the explanation, it is becoming clear that, even in these simple *in vitro* systems, we can see complex pharmacological behaviour that would be missed by studying drug actions only at the chemical level.

So, how can we study complex systems in the lab? We have intact animals and man at the top, and then come isolated, perfused, organs, then pieces of tissue, with their cellular architecture-intact, suspended in organ baths, then cells in tissue culture, then homogenized cells and, finally, purified proteins. Reductionism in biology merely replaces one type of complexity by a different kind of complexity. No one level is more reliably informative then any other. So I strongly believe that pharmacology needs to be studied at all levels, the choice of level being dictated by the nature of the question being asked. The choice of level for studying the pharmacology of complex systems in the first instance is the intact tissue bioassay. The attractiveness of these bioassays is that they can be driven chemically and physically in as many ways as our imaginations can conceive and yet still remain, potentially at least, mathematically tractable and analysable. So, I believe that intact-tissue bioassays, which were rejected by the modern industry as yesterday's technology, still have an important future.

Alfred Nobel died in 1896. In his will, he commanded that the interest from his estate should be distributed in the form of prizes to those who 'shall have conferred the greatest benefit on mankind' (Sohlman, 1983). In his recent book on the history of medicine, Roy Porter summarized his views about the social contributions of medical practice under the title 'The Greatest Benefit to Mankind' (Porter, 1997). There can be little doubt that the discoveries and inventions of new drugs have made a significant contribution to that benefit. In this essay, I have tried to explain why I think that the invention of new drugs could still have an exciting future and hope they may continue to contribute to the 'benefit of mankind'.

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