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A Life in New Drug Research

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Part 1: Recollections

My interest in drug research began in 1950. The University of Glasgow had acquired by Act of Parliament the old Glasgow Veterinary College that had been a private company for years which was housed in old, academically unsuitable buildings in downtown Glasgow. The University renamed it the University of Glasgow Veterinary School and tried to recruit new staff. By an extraordinary series of coincidences, I was given the chance to build a new Physiology Department from scratch. In addition to building new teaching laboratories, I was able to build my own research lab and workshop. In those days we had to build our own equipment. Also, working in a veterinary environment, I had exceptional access to animals for experimental use. I cannot now remember how it happened but two surgeons from the University – George Smith, cardiac surgeon, and Adam Smith, GI surgeon, discovered these 'resources' and joined me in my lab every week. Indirectly, I got the ideas from working with George Smith that would lead eventually to the 'beta-blocker' story. Curiously, my work with Adam Smith would eventually lead on to the 'H2 antagonist' story.

George Smith was interested in the treatment of angina pectoris well known to be the result of atheroma in which fatty deposits in the vascular intima narrowed the lumen of the coronary arteries. I too had in interest in angina pectoris and the sudden death associated with that syndrome due to the precipitation of ventricular fibrillation. As a young medical student I watched my father die suddenly after a minor car crash. My father had had angina for many years and I remember wondering if it was enhanced sympathetic drive to the heart, the reaction to 'fight, flight or fright' that had precipitated ventricular fibrillation. 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 chest pain. With the onset of an attack, patients would stop, fumble with their pillbox 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 act of stopping to find their pillbox may have been enough! Nevertheless, industrial pharmacologists invented new drugs that were selective coronary vasodilators in healthy anaesthetised 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. George Smith had trained with Beck before he joined me. He wanted to develop an idea that he had 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 anaesthetised dogs is tied, 9/10 animals developed ventricular fibrillation within 2 hours. However, when the dogs were put in a high pressure chamber filled with 100% oxygen at 2 atmos pressure, then only 1/10 dogs fibrillated in the same time period. Of course I knew that haemoglobin was fully saturated with oxygen at the normal atmos. pressure of a fifth of an atmos **(1)**. 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 were 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

generalised 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 twenties. 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' **(2)** edited by Drill. To my excitement, I found a chapter on Adrenergic Drugs by Ray Ahlquist. He was able to use this opportunity to expound his own highly original idea. He had had great trouble getting his original work and ideas accepted but his paper was eventually published in 1948 in the American Journal of Physiology largely because the Editor, W.F.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 textbook for doctors. 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 that 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 that 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. Remember that in the 1950's 'receptors' were mere concepts. Ahlquist, in his autobiography **(3)**, 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.

By this time it had become clear to me that what I wanted was a selective 'antagonist' active only at adrenaline's 'beta-receptors'. The structure of isoprenaline seemed to point to how I might try to invent a competitive antagonist in theory but reduction to practice required funding. I had got to know the 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 with excitement at the prospect and moved south in June 1958.

I was assigned a new colleague, the chemist John Stephenson, and a technician Brian Horsfall to work on the new 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 the challenge to John Stephenson 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 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 terminal nitrogen atom. Iso-

prenaline 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 didn't! We stayed with the nitrogen end of the molecule and tried replacing the isopropyl group with larger and larger phenyl-ethyl and related groups. It would be an understatement to say that, chemically, we did not really know what we were doing!

Then, early in 1959, we came across a paper that described the properties of the dichloro-benzene analogue of isoprenaline (known as DCI). Powell & 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 **(4)**. Stephenson quickly synthesised DCI for me and to my astonishment was as potent an agonist as isoprenaline on my cardiac pacemaker (Langendorff) preparation! As we had no idea what this meant, the compound was shelved. Meanwhile, I was busy trying to develop a new biosassay. 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 anti-vivisection emotions, but I drew a line at killing kittens! From a practical point of view, kitten muscles were ideally relatively long and thin. 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, I got another surprise. DCI did not augment the force of contractions but now antagonised 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 spatial 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 **(5)**. This compound, which was given a house name 'nethalide' and eventually given an approved name of pronethalol, was the first beta-receptor antagonist (popularly known as 'beta-blockers') to be tested clinically before the results of a two-year toxicity test showed it to be a weak carcinogen. Beta-naphthylamine was known to be a potent carcinogen. Pronethalol is a beta naphthyl derivative of ethylamine. So, two years after going in to man it was replaced by propranolol, an alpha naphthyloxy methyl derivative of ethylamine. This compound was found to be 10x more active and less toxic than pronethalol and not carcinogenic.

The transfer of pronethalol from lab animals to man took place before post-thalidomide regulations were introduced. In the event, Professor Dornhorst, physiologist and physician, spent two days at ICI with me while I demonstrated live to him all the experiments that were at the basis of our claim. This included a demonstration of a kind of 'hind limb' paresis that developed in conscious beagles. Dornhorst – "These dogs look cold to me – have you measured their temperatures?" They hadn't – red faces all round in the local team! When we warmed them up by exercise the hindlimb 'paresis' disappeared. We learned later that these dogs did indeed have a temperature-control problem due to widespread skin vasodilatation.

Dornhorst moved rapidly into man. The first subject, a senior colleague, was given the drug intra-arterially and showed that it completely blocked the local vasodilatation produced by intra-arterial isoprenaline, Then another colleague was given the drug intravenously that again completely blocked the effects of intravenous isoprenaline. Dornhorst then tested pronethalol on the cardiac responses to exercise. Two medical students volunteered to exercise maximally on stationary bicycles. One was very fit – a cross-country runner. The other was overweight and decidedly not very fit. Pronethalol had very little effect on either resting or exercising pulse rate and he was physically embarrassed by the drug. His unfit colleague had a very high pulse at rest that became almost uncountable during exercise. The drug not only greatly reduced both his resting and

exercising pulse rates but he felt much more comfortable during exercise. After this Brian Prichard, clinical pharmacologist and physician at University College London began his clinical studies in patients and in addition to showing some relief from angina he made the seminal observation that the drug produced a gradually (over weeks) a non-posture dependent reduction in the blood pressure of hypertensive patients.

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. Driven by the anomalous responses to DCI, I was also beginning to learn some pharmacology. I learned that 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 1950's, Stephenson **(6)** and Ariens **(7)** 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. As I've described earlier, on my first project, before I understood this theory, I was surprised by the differing responses of the assay tissues. Curiously, on my next project changing the assay again changed the response.

When the inventive phase of my 'beta blocking' programme was coming to an end I began looking around for a new project Then I remembered the work I had done 10 years earlier with Adam Smith. When he joined me in my new lab at the Vet School he had just spent a year with Feldberg at the NIMR in Hampstead 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 **(8)**. Through Adam Smith, I heard about his colleague, Andrew Kay's work on his '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 peptic ulcers **(9)**. 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. This work came back to my mind as my beta-blocker programme was reaching the clinic. Having achieved the 'beta blocker' target, I began to wonder "Could histamine too have its beta receptor equivalent?".

However, although I had known for years that histamine was a potent pharmacological stimulant of gastric acid secretion, I soon came realise that by the mid 60's that there was very little interest in histamine as a physiological mediator of food-stimulated gastric secretion. By 1964, Rod Gregory at Liverpool had extracted pure gastrin from stomach tissues of abattoir pigs and showed that it was a very potent stimulus of gastric acid secretion. His colleague George Kenner, Professor of Chemistry at Liverpool, showed that gastrin was a small, 17 amino acid, protein that he confirmed by its total synthesis. 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 **(10)**.

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 **(11)**. 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 was right, I might have found a way of inhibiting the oversecretion 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.

I joined Smith, Kline & French Laboratories at Welwyn Garden City with the specific remit to try to invent an H2 receptor antagonist. 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. Our initial bioassay was the lumen-perfused isolated guinea pig stomach that was driven to secrete acid by histamine in the perfusion fluid. Graham Durant started by systematically replacing, one at a time, all of the hydrogen atoms with a methyl group. To our excitement, we found that one of these, 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, the equivalent of isoprenaline at beta receptors. I knew then that I was on the right track. However, 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 didn't. It took three years of slow and difficult chemistry before I realised my error. The breakthrough came when we developed a new in vitro assay, the isolated, electrically-stimulated guinea pig right atrium. In evaluating this assay, we retested many of our old compounds. Imagine the thrill of finding that one of them, them, a compound that replaced the terminal amino group with a guanidine group, which was a full agonist on acid secretion, turned out to be a weak antagonist to histamine on the new atrial assay. The guanidine analogue of histamine was to H2 receptors what DCI was to beta receptors. Once the 'dog saw the rabbit', my colleague Robin Ganellin led the subsequent development. Lengthening the chain of atoms that joined the guanidine group to the imidazole ring produced more potent compounds. Eventually, a compound was made that met our minimum requirements for development – the 4-methyl imidazole was joined to a thiourea group by a 4 carbon chain. We called it burimamide and it was the first H2-receptor antagonist to go into man Metiamide followed where the electron-withdrawing effect of the side chain was insulated from the imidazole ring by replacing a side chain carbon with an isosteric sulphur atom. Eventually, the potential toxicity of the thiourea group was replaced by a cyano guanidine group to produce a compound that was eventually marketed as cimetidine **(12)**. 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 and co-workers 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 **(13)**. 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 termi-

nal 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. 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. Indeed, I now share with Malcolm Boyce a licence to compound YF476 that is a highly effective, orally active, gastrin antagonist. In the last few years there has been growing unease among gastroenterologists that drug-induced increases in the blood levels of gastrin, hypergastrinaemia, 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 **(14)**. 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 personal examples, 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 generalise a strategy for drug research. A drug research strategy is the set of principles that help you 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 some 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 seemed till recently 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 antagonise 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.

Part 2: Projections

The background of many of the prejudices I will express in this section has been my experience of research working in small groups in ICI (now Astra Zeneca) and SK&F, managing a large Research Division at the Wellcome Foundation, and in 1982 I was given the opportunity to set up my own independent group within King's College London. I will briefly expand on this latter experience towards the end of this section.

My first prejudice is about the need to distinguish between invention and discovery. Discovery is about exposing the mysteries of nature, of what already exists. Invention is about creating something that never existed before. Discovery is the major activity in academic, University, research. Invention is the major preoccupation in industrial research. Discovery is about the excitement of travelling. Invention is about the satisfaction of arriving when it becomes 'fit for purpose'. Today, research grant applications are invariably made by a group with further discoveries as the objective. I cannot imagine a grant application by an individual to make an invention. The point that I want to make is that, psychologically, inventors and discoverers are different kinds of people with different needs and management. Perhaps we need the equivalent of football scouts to scour the academic research labs for frustrated individuals with ideas that they cannot get funded to do. I know they are there. When I get the chance, I always ask bright investigators working in a team "Are you really working on what you would like to do ?" I've had some interesting answers!

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 organisations 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. So 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 compounds 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-synthesised, 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 incredible 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. Now, as far as I know the new drugs that the pharmaceutical industry is seeking, to treat disorders such as asthma, cancer and dementia 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 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 acetylcholine 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 organised by chemical convergence based on potentiating interactions will need pharmacological convergence to manage them effectively. An interesting example has been reported by Buchsbaum and colleagues **(15)**. He measured the growth of pancreatic cancer cells implanted subcutaneously in nude mice. He treated the mice with 3 agents; gemcitabine, C225 an epidermal growth factor inhibitor and local irradiation. Individually, each therapeutic modality had a characteristic but short-acting effect. In paired combinations, the effects were greatly amplified but eventually reversed by tumour growth. However, the combination of the three treatments produced a 'cure'. Could this principle also apply to man?. 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 combinations.

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. 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 **(16)**. 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 homogenised 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 as part of an armoury of investigation.

Fortunately, the incredible slowness of the old techniques of bench chemistry, screening bioassays and the iterative methodology can now be replaced by some very fast new technologies. Although I have shown that an iterative strategy can successfully lead to the discovery of new drugs, the process was very slow. Although only several hundred compounds at most need to be synthesised, the process can often take several years. While the main rate-limiting step is the bench synthesis steps, the bioassays I have described need significant quantities of pure compound to construct families of dose-response curves. Brian Warrington originally led the team at GSK to tackle these problems. They have developed a new technology known as 'closed loop microfluidics'. In this technology, chemical reactions take place in a glass-based flow reactor sufficiently small to ensure short mixing and reaction times (ca 1 second) and minimal reagent demand (ca 1 microgram). Coupled to a micro-scaled LC-MS the module can generate sub microgram quantities of pure compound in a couple of hours for delivery to coupled assay systems. This technology is developing rapidly and the latest position can be obtained from my old colleague Andy Vinter at Cresset (Welwyn Garden City, UK). Vinter himself has developed his molecular field technology to such a level that he can now use it for screening data bases **(17)**. I have no doubt that these new technologies could revolutionise the new drug invention process.

Finally, I want to add a note on managing new drug invention research groups. I mentioned that in 1988 I was given the opportunity, by the generosity of Johnson & Johnson, of setting up my own research group within the ambit of King's College London. My agreed game plan was that I would control the research head-count to 20 plus 5 administrative posts including me. By controlling the head-count I know that I would effectively control the annual budget. All of the scientists I employed were Ph.D's or equivalents. There were no technicians. We concentrated on one project at a time. We worked like a football team, each of us know our role. No one spent their time sitting in offices or attending 'management meetings'. We met as a group when we wanted to share with each other our progress. Of course we had to prepare 6-monthly reports for J&J but these came straight out from our daily lab notebooks. All I can say is that this plan worked well for the next 20 years until it was closed some time after I had to retire. I really believe that such a plan could work in a modern industrial environment where the primary phase of research would be taken care of by as many of these independent groups as the Company could afford.

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". In his recent book on the history of medicine **(18)**, Roy Porter summarised his views about the social contributions of medical practice under the title "The Greatest Benefit to Mankind". 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'.

Conflicts of interest

JB has no potential conflicts.

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