basic research remains a feature of academic institutions would not then be wholly lost to pragmatism.

REFERENCES

1 McGregor A. Swiss health insurance curb on pharmaceuticals. *Lancet* 1994; **344:** 321–2.
2 Court C. SmithKline Beecham buys US company. *BMJ* 1994; **309:** 629.
3 Pharmaceuticals from plants: great potential few funds. *Lancet* 1994; **343:** 1513–5.
4 Reynolds T. Tropical rain forest conservation tied to drug development (news). *J Natl Cancer Inst* 1991; **83:** 594–6.
5 de Stevens G. Serendipity and structured research in drug discovery. *Prog Drug Res* 1986; **30:** 189–203.
6 Cox B. Strategies for drug discovery: structuring serendipity. *The Pharm J* 1989; **243:** 329–37.
7 Sun E, Cohen E. Computer assisted drug discovery—a review. *Gene* 1993; **137:** 127–32.
8 House of Lords Select Committee on Priorities for the Science Base 1993. Evidence of the Association of the British Pharmaceutical Industry.
9 *Scrip* 1994; **1927:** 6.
10 Vane J. In: Frangsmyr T (ed). Nobel Lectures. Physiology and medicine 1981–1990. World Scientific Publishing Co 1993, 141–4.
11 Black J. In: Frangsmyr T (ed). Nobel Lectures. Physiology and medicine 1981–1990. World Scientific Publishing Co 1993, 413–7.
12 Garaghan H. Reasonable pricing may be dropped from NIH deals. *Nature* 1994; **370:** 238.
13 Association of British Pharmaceutical Industry. Annual Review 1993–4.

Proc. R. Coll. Physicians Edinb. 1995; **25:** 5–24

Leading Article

THE DISCOVERY OF DRUGS

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EARLY MEDICINES

Anthropologists are unaware of any human culture that did not instinctively, and hopefully, turn to natural products in an attempt to alleviate its suffering. Thus, it appears that Man always has taken 'medicines' in the hope of putting right what has gone wrong with him, of enabling him to escape mentally from depressing surroundings, or of heightening religious or mystical experiences. And so we can imagine the first *Homo sapiens*, or even their ancestors, grubbing around among the leaves and roots in the hope of finding a cure for their ills. Sooner or later of course, by sheer coincidence, something that seemed to help would be found, and the treatment would then become part of folklore.

As long ago as 30,000 BC, Australian aboriginals probably knew of the medicinal properties of acacia, eucalyptus oil, and the corkwood tree (which contains hyoscine). In 2700 BC, the Chinese emperor Shen Lung described the uses of rhubarb, tea, and Ephedra species (which contain ephedrine). The medicinal uses of opium, pomegranate (for round worm infestation), powdered liver (for night blindness—contains vitamin A), *Hyoscyamus*, castor oil, figs and senna are described in an Egyptian papyrus of 1550 BC found by Gorg Ebers at Thebes over 120 years ago. Theophrastus in Greece in about 320 BC added mustard, tar ointments, male fern (for tapeworm), aloes and the Mediterranean mandrake to the list. The last, despite its reputedly magical properties, contains no more than hyoscine and related alkaloids. Dioscorides, in the first century AD, collected medicinal plants during his travels as a surgeon in Nero's army. He described 500–600 such plants in a well documented herbal but, generally speaking, there was nothing new compared with those of his predecessors. The same seems to be the case even in the writings of Nicolas Culpepper, the famous 17th century herbalist. In the 12th century, Arabian medicine made use of colchicum for gout and Roger of Salerno described the use of burnt sponge (containing iodine) for the treatment of goitre. In the 16th century, Dutch sailors discovered that lemons prevented the development of scurvy and this concept, using limes instead of lemons, was taken up by the Royal Navy. Hence British sailors, and subsequently all of us, became 'limeys' to our American cousins.

Historically, the practice of medicine was often tied up with religion; many ancient physicians were also priests. The religious aspects led to a number of rather bizarre philosophies, amongst which was the Doctrine of Signatures which held that a merciful god had provided medicinal plants with a sign, or signature, in their appearance, which would indicate their curative properties to the cognoscenti. Hence hepatica (with liver-shaped leaves) and pulmonaria (with alveolar-like leaf markings) were erroneously adduced to be useful in liver and lung diseases respectively. Generally speaking, the doctrine was a failure although

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aspirin might be said to be derived from a related philosophy which held that God had placed the cure near the cause. It is a well known story that in 1763 the English cleric, the Rev Edward Stone, felt that a treatment for malaria (the ague) must be found in damp swampy areas, since such areas seemed to give rise to the condition. Since willow trees (family: *Salicaceae*) grow in such areas, he gave his suffering parishioners an extract of white willow tree bark, and it seemed to work, or at least to control the fever. The salicin in the bark is a derivative of salicylic acid. It is not of course an antimalarial, but the observation was an early trigger in the eventual synthesis of acetylsalicylic acid (aspirin) by the Bayer company in Germany in 1899.[1] Other important naturally-occurring drugs derived serendipitously from folk-lore included digitalis, the value of which in heart failure was not properly established until 1785 by William Withering in Birmingham. Some remedies were brought back to Europe from afar by explorers. For example, cinchona for malaria and ipecacuanha for dysentery were brought from the Americas in the 17th century, and calabar beans (containing physostigmine) from Nigeria in 1840. The leaves of *Erythroxylon coca* have been chewed by the inhabitants of the high Andes mountains for centuries. Cocaine was isolated from these in 1860 and introduced as a local anaesthetic by Koller in 1884. Ergot had been used by European midwives since the 16th century, but was introduced into official medicine only in 1808. The South American arrow poison curare was described to Elizabeth I of England by Sir Walter Raleigh. It was used in physiological experiments throughout the 19th century, and Claude Bernard determined its site of action in 1856. However, its value as a muscle relaxant during surgical anaesthesia was not realized until 1942, when Griffith and Johnson in Montreal first described its use as such.

The most important discovery in the first half of the 19th century were the general anaesthetics; nitrous oxide by Humphrey Davy in London, ether by William Morton in Boston, and chloroform by James Simpson in Edinburgh. General anaesthesia, as a technique, may be said to have begun in 1846 with Morton's demonstration of the efficacy of ether in the Massachusetts General Hospital. In the UK, ether was first used in the Dumfries Royal Infirmary as soon as the news was brought back from Boston. The second half of the 19th century saw the development of the germ theory of infectious diseases, which led both to the production of immunological preparations and to the synthesis of chemotherapeutic agents. Jenner had demonstrated successful immunization against smallpox by vaccination with cow pox in 1798, but it was Pasteur's work on rabies vaccine in 1880 that set the scene for all future developments. Around this time, Paul Ehrlich came to believe that aniline dyes might exert selective toxicity against parasites, and in 1891 he demonstrated that methylene blue had some effect in the treatment of malaria. This observation led, early in this century, to his discovery of the organic arsenical salvarsan 606, its efficacy against syphilis, and subsequently to the discovery of synthetic antimalarials and sulphonamides. This was followed by the development of antibiotics after the observations of Fleming and Florey and Chain with penicillin.

The concept that synthetic chemicals might have therapeutic benefit was therefore growing towards the end of the 19th century, and was reinforced by the discovery of the effects of chloral hydrate (1860), amyl nitrite (1860), glyceryl trinitrate (1870), aspirin (1879), paraldehyde (1880), phenacetin (1880), and barbiturates (1900). But despite the progress, by the end of the 19th century, the useful

drugs out of the 857 listed in the *British Pharmacopoeia* of the time, numbered no more than 20 or so; amyl nitrite, general anaesthetics, atropine, bromides, cocaine, digitalis, ephedrine, ergot, ipecac, morphine, physostigmine, quinine and salicylates, were the most important. Many of these are of very ancient vintage. Sir Henry Dale, writing in 1943, records his desperate disappointment as a medical student around the turn of the century, on learning how poorly effective most drugs of the time were, and how little was known about how even the effective ones worked.[2] For descriptions of early history, articles written by Dale,[2] Gaddum,[3] Keele[4] and Midgley[5] may be consulted.

THE MODERN ERA

The rapid growth of knowledge in physics, organic chemistry, physiology and biochemistry beginning in the 19th century led to two important developments which revolutionised therapeutics and produced the drug explosion of recent years. These were (i) the techniques of scientific experimentation and scientific logic, and (ii) the concept that biological processes are mediated and modulated by endogenous body chemicals, and that disease (other than infection) frequently arises from a dysfunction on the part of, or an imbalance between, these chemicals. Rectification of the imbalance by exogenous chemicals then became a logical goal, which led to the development of pharmacology as a science in its own right. Clearly there is more motivation to discover logical treatments when it is known, for example, that mental diseases are probably a consequence of some kind of derangement in the brain's neurotransmitter systems rather than to the patient being possessed by devils. Familiar examples of such chemical derangements and their logical treatments include (1) Parkinson's disease, arising from dopamine deficiency in the nigro-striatal pathway, and its treatment by restoring (levodopa), mimicking (e.g. pergolide), or reducing the destruction of (e.g. selegiline) the deficient dopamine, and (2) diabetes mellitus, arising from insulin deficiency or from its inadequate function, and its treatment by restoring the missing hormone, enhancing its release from the B cells of the pancreatic islets (e.g. glibenclamide) or facilitating the activity of what remains (e.g. metformin).

At the same time, development of new skills is still accelerating in protein and carbohydrate chemistry, in immunology, in new imaging techniques in electron microscopy, in electrophysiological recording, in computing and in computer graphics, and following upon the work of Watson and Crick, in recombinant DNA technology and other aspects of cell and molecular biology. Together, these have created a revolution in the medical and biological sciences, including drug development. This revolution is sweeping along like a whirlwind, with the result that faith in the development of Paul Ehrlich's 'magic bullets', that is, specific drugs for each and every one of man's diseases, is thought by many, probably over-optimistically, to be just around the corner.

In general, academic scientists make relatively few direct excursions into drug discovery, but spend much of their research time using drugs as tools, in attempts to discover endogenous chemical mechanisms that drive the body systems in health and disease. Four of many recent and unrelated examples of discoveries in academic departments about endogenous chemical control mechanisms follow. (1) The blossoming discoveries relating to endogenous nitric oxide and its mechanisms of action in controlling the calibre of blood vessels, in functioning as a

neurotransmitter both peripherally and centrally (including its possible roles in learning and memory), in its pathological roles in neuronal death after a stroke, and in its use by macrophages and neutrophils to kill invading bacteria.[6] To some extent carbon monoxide may play similar roles.[7] (2) The discovery by workers at Aberdeen University of an endogenous brain chemical, now called anandamide, that selectively stimulates cannabinoid receptors.[8] Interestingly, the endogenous brain enkephalins, which stimulate morphine receptors, were discovered by Kosterlitz and Hughes in the same department 15 or so years ago. (3) The discovery of nerve growth factors, a recent example being the glial cell line-derived neurotrophic factor that, at least in culture, selectively causes dopamine-containing neurones to survive and grow.[9] (4) The unravelling of genetically programmed cell death (apoptosis) that occurs when removal of certain cells is a desirable event (e.g. in a developing embryo). This process, first described by Alexander Currie in Edinburgh,[10] is under strict endogenous chemical control.[11]

Knowledge of these chemical control systems does not directly provide therapeutic answers, but it suggests the discovery and development of exogenous chemicals (i.e. drugs) for mimicking, stimulating, or antagonising the appropriate system when disease makes this appropriate. The pharmaceutical industry, with its immense resources and manpower, is skilled in synthesizing, designing and developing the most appropriate, most selective, and least toxic drug possible under the prevailing circumstances. The division of labour between academe and industry is not absolute; important forward leaps of an academic nature have certainly been made by industrial scientists, and some new drugs have been discovered in Universities. An example is the muscle relaxant atracurium, invented in a university but developed by industry.[12] Furthermore, when industry has found what appears to be the best drug available for a particular purpose, its first clinical trials are frequently conducted by the clinical pharmacology departments of universities. The distinction between basic academic research and applied industrial research is blurred and is becoming increasingly so; perhaps in no other discipline is the collaborative approach between academe and industry more obvious and fruitful than it is in drug design.

CHEMICAL CONTROL OF BODY PROCESSES

The new knowledge that is most important to drug design is that related to cell membranes and the processes of transmembrane signalling. Embedded in the lipid cell membrane are a myriad specific protein or glycoprotein molecules, many of which serve as the receptor sites for the chemical messengers arriving in the blood stream from glands, or released locally from nerve endings. Individual cells can respond to a particular chemical messenger only if they possess the specific receptors. Thus, for example, the smooth muscle of arterioles responds to circulating angiotensin II but skeletal muscle does not, because only the former possesses angiotensin receptors.

The basis of the transducing property of the receptor proteins is their ability (like all proteins) to undergo a change in shape, that is a conformation change, when acted upon by specific small molecules. Indeed, this property of proteins is the basis of all living processes. For a fascinating insight into proteins and their functions a small monograph by Max Perutz, who has himself contributed hugely to the field, may be consulted.[13]

Fortunately for therapeutics, cell surface receptors for a particular endogenous chemical messenger are hardly ever, perhaps never, of a single type. This has been known for many years—long before the advent of molecular biology. Witness, for example, Dale's observation in 1914 that, although the chemical messenger, the agonist, for what he called cholinoceptors is always acetylcholine, the receptors themselves are not all identical. Dale designated two subtypes, nicotinic cholinoceptors and muscarinic cholinoceptors, because, while both responded to acetylcholine, the former responded selectively to nicotine (from tobacco) and the latter to muscarine (from the toadstool, *Amanita muscaria*). A further fortuitous arrangement of nature is that generally speaking, although not absolutely, one subtype of receptor predominates in one type of tissue, and another in another. A useful degree of selectivity of drug action is therefore afforded. Synthetic agonists or antagonists may therefore be made in the laboratory, or obtained from other sources, that will act upon one of Dale's subtypes but not the other. In the particular field of acetylcholine receptors, antagonists are on the whole therapeutically more important than agonists. Illustrative and well known examples are that atropine blocks muscarinic acetylcholine receptors in salivary glands and dries up secretions, but does not paralyse skeletal muscle, whereas tubocurarine blocks nicotinic acetylcholine receptors in skeletal muscle producing muscle relaxation for surgical anaesthesia, but does not dry up secretions. Current molecular biology shows that genes are present to specify 5 subtypes of muscarinic receptors (M_1–M_5), and an as yet unknown, but large, number of subtypes of nicotinic acetylcholine receptors, so that further selectivity of drug action is probable in the near future. For example, an atropine-like bronchodilator that does not cause tachycardia, dry up secretions, nor cause difficulty in evacuating the bladder might be designed and developed.

Another familiar example that predates molecular biology is Ahlquist's demonstration in 1948 that receptors for adrenaline and noradrenaline (adrenoceptors) are of two subtypes which he designated α-adrenoceptors and β-adrenoceptors. Lands and his co-workers[14] subsequently showed that β-adrenoceptors may be further subdivided into β_1- and β_2-adrenoceptors. Isoprenaline is a more useful bronchodilator than adrenaline, partly because it selectively acts on β-adrenoceptors, but it still produces tachycardia because the cardiac adrenoceptors also belong to the β-subtype. However, the cardiac adrenoceptors are mainly of the β_1-subtype, whereas the bronchial adrenoceptors are mainly of the β_2-subtype. Hence, salbutamol, terbutaline and salmefamol, for example, are more selective bronchodilators than is isoprenaline because they act mainly on β_2-adrenoceptors. Conversely, atenolol is a cardioselective antagonist because it blocks only the β_1-subtype of adrenoceptors. Propranolol is non-selective because it blocks all subtypes of β-adrenoceptors. The techniques of molecular biology, coupled with those of pharmacology, have shown that this subclassification of adrenoceptors is much oversimplified, as there are at least 10 subtypes of adrenoceptors—7 alpha subtypes and 3 beta subtypes (Table 1). Table 1 lists the various subtypes of the most important receptors for many known endogenous ligands. Most of this knowledge, which is still expanding, has also been derived from a combination of molecular biology and classical pharmacology techniques. Generally speaking, receptor nomenclature is chaotic because it has grown up with the development of the subject. It may now be too late to produce a comprehensive and systematic nomenclature. Compounds, not necessarily thera-

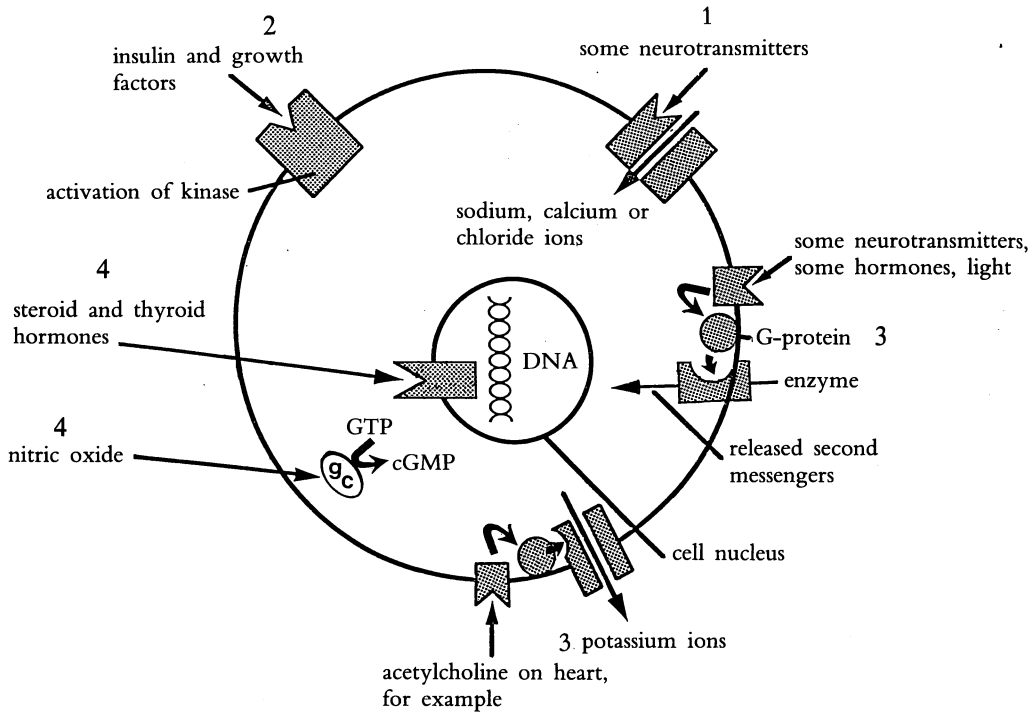

FIGURE 1

Four mechanisms through which a chemical messenger (the agonist) instructs the cell. (gc is the enzyme guanylyl cyclase).

peutically useful drugs, that act at least with a good degree of selectivity on each type of receptor are available in most instances.[15]

Although there is a multiplicity of receptors, there are only four main mechanisms through which the released chemical messenger, the agonist, instruct the cell.[16] These are illustrated in Fig 1 and are enumerated below, the numbers corresponding to those in Fig 1. Table 1 lists the mechanism through which each membrane receptor subtype functions.

1. Receptor-coupled ion channel. These receptors are made up of 4 or 5 separate proteins or protomers in a ring around a central pore that passes through the membrane. In the resting state, the pore is closed. When the specific activating chemical, in every case a neurotransmitter, interacts with its binding site on one of the protomers a conformational change occurs which is transmitted throughout the receptor complex. The result is that the ion channel opens so that particular ions to which the open channel is permeable, flow through it down their concentration gradient. The ion flux changes the electrical potential across the membrane and this causes the cell to respond. The main receptors of this type are the nicotinic receptors, some subtypes of ATP receptors, and some subtypes of glutamate receptors which are cation (mainly Na^+ and/or Ca^{2+}) selective, and glycine receptors and the A subtype of gamma aminobutyric acid ($GABA_A$) receptors which are chloride ion selective.

2. Receptors with intrinsic enzymatic activity. In these cases, the extracellular messenger, on combination with its receptor, causes a conformational change in

TABLE 1

Membrane receptors for endogenous ligands

Receptors	*Subtypes (effector pathway denoted in brackets)*
Acetylcholine receptors	M_1(3b), M_2(3c or d), M_3(3b), M_4(3c), M_5(?), N_{muscle}(1), $N_{neuronal\ ganglionic}$(1), $N_{neuronal\ \alpha7}$(1) M = muscarinic; N = nicotinic
Adrenoceptors	α_{1A}(3b), α_{1B}(3b), α_{1C}(3b), α_{1D}(3b)α_{2A}(3c) α_{2B}(3c), α_{2C}(3c), β_1(3a or f), β_2(3a), β_3(3a)
Angiotensin receptors	AT_1(3b or c) AT_2(?)
Atrial natriuretic peptide receptors	ANP_A(2) ANP_B(2) (cyclic GMP ↑ in both cases)
Bradykinin receptors	B_1(3b) B_2(3b)
Calcitonin gene related peptide (CGRP) receptors	CGRP receptors (3a) Subtypes not yet clear
Cannabinoid receptors	CB_1(3c) CB_2(?)
α-Chemokine receptors	$IL8_A$(3b) $IL8_B$(3b)
β-Chemokine receptors	$MIP1_\alpha$(3b) MCP1(3b)
Cholecystokinin receptors	CCK_A(3b) CCK_B(3b)
Dopamine receptors	D_1(3a) D_2(3c, d, or e) D_3(3e) D_4(3c) D_5(3a)
Endothelin receptors	ET_A(3b) ET_B(3b)
Excitatory amino acid receptors (glutamate)	NMDA(1) AMPA(1) kainate (1) metabotropic*(3b or c) *possibly 7 subtypes of metabotropic receptors
GABA (gamma amino butyric acid) receptors	$GABA_A$(1) $GABA_B$(3c, d or e)
Galanin receptors	Subtypes not yet clear (3c, d or e)
Glycine receptors	Subtypes not yet clear (1)
Histamine receptors	H_1(3b) H_2(3a) H_3(?)
5-Hydroxytryptamine (serotonin) receptors	5-HT_{1A}(3c) 5-HT_{1B}(3c) 5-HT_{1D}(3c) 5-HT_{1E}(3c) 5-HT_{1F}(3c) 5-HT_{2A}(3b) 5-HT_{2B}(3b) 5-HT_{2C}(3b) 5-HT_3(1) 5-HT_4(3a) 5-HT_{5A}(?) 5-HT_{5B}(?) 5-HT_6(3a) 5-HT_7(3a)
Leukotriene receptors	LTB_4(3b) LTC_4(?) LTD_4(3b)
Melatonin receptors	Subtypes not yet clear (3c)
Neuropeptide Y receptors	Y_1(3c) Y_2(3c)
Neurotensin receptors	Subtypes not yet clear (3b or c)
Opioid receptors	μ(3c, d or e) δ(3c, d or e) κ(3c, d or e)
Platelet-activating factor (PAF) receptors	Subtypes not yet clear (3b)
Prostanoid (prostaglandin) receptors	DP(3a) FP(3b) IP(3a) TP(3b) EP_1(3b) EP_2(3a) EP_3(3b or c)
P_1-purinoceptors (adenosine)	A_1(3c, d or e) A_{2A}(3a) A_{2B}(3a) A_3(3c)
P_2-purinoceptors (ATP)	P_{2X}(1) P_{2Y}(3b) P_{2Z}(1) P_{2T}(1 or 3c) P_{2U}(3b)
Somatostatin receptors	$SRIF_{1A}$(3c) $SRIF_{1B}$(3c) $SRIF_{1C}$(3c) $SRIF_{2A}$(?) $SRIF_{2B}$(?)
Tachykinin receptors	NK_1(3b) NK_2(3b) NK_3(3b)
Vasopressin and oxytoxin receptors	V_{1A}(3b) V_{1B}(3b) V_2(3a) OT_3(3b)
Vasoactive intestinal peptide (VIP) and related peptide receptors	VIP_1(3a) VIP_2(3a) GRF(3a) PACAP(3a) Secretin (3a) GRF = growth hormone releasing factor PCAP = pituitary adenylyl cyclase activating polypeptide

The numbers in brackets refer to the mechanisms illustrated in Fig 1. 3a = stimulation of adenylyl cyclase with a consequent increase in cyclic AMP production. 3b = stimulation of phospholipase C with the production of inositol trisphosphate (IP_3) and diacylglycerol (DAG). 3c = inhibition of adenylyl cyclase. 3d = opening of K^+ channel. 3c = closure of Ca^{2+} channel. 3f = opening of Ca^{2+} channel. (Data from Watson and Girdlestone[15])

the protein that results in activation of an internally directed enzyme that is an integral part of the same protein complex as the receptor. Examples of the extracellular chemical signals include atrial natriuretic peptide, the receptors for which are directly coupled to a guanylyl cyclase enzyme, and polypeptide growth factors or mitogens that control cell proliferation and differentiation. Insulin acts on a similar type of receptor. The enzyme component of the polypeptide growth factor and insulin receptor complexes is a kinase, a phosphorylating enzyme, that transfers a phosphate to another protein. In some instances, interaction with the receptor causes the receptors to form pairs which phosphorylate each other. The activated couplet then phosphorylates an intracellular protein. An important pathway involved in cell proliferation and differentiation is the mitogen activated kinase (MAP) pathway. MAP kinase enzymes are themselves activated by phosphorylation by another kinase (MEK). The stimulation of receptor kinases by a growth factor or mitogen activates MEK. MEK activates MAP kinases which in turn phosphorylate and thereby activate a number of transcription factors in the nucleus with the result that appropriate protein synthesis is stimulated. MAP kinase activity appears to be involved in the thickening of the arterial walls in hypertension and of the bronchi in chronic asthma. Hence, drugs that inhibit MAP kinases, the crystal structure of one of which is now known,[17] may provide a means of reducing the contribution of smooth muscle hypertrophy to these diseases.

3. G-Protein coupled receptors. A G-protein is a membrane protein that binds guanosine triphosphate (GTP) when activated. The activated G-protein then acts either as a kind of molecular switch that switches enzyme action on or off by altering the conformation of the enzyme, or it directly opens or closes a membrane ion channel. In the resting state, the G-protein is bound to GDP. When the extracellular chemical messenger binds to its receptor, the conformation change induced in the receptor protein enables it to interact with the G-protein which then gives up its GDP in exchange for GTP. The part of the G-protein combined with GTP dissociates from the rest, and usually it is this part that then diffuses to the target ion channel or enzyme. Occasionally, it is the GTP-free part of the G-protein that functions in this way. Since the G-protein is itself a GTP-ase, activity is terminated as soon as the G-protein converts the bound GTP to GDP by removing a phosphate.

The enzymes known at present to be activated by G-proteins are phospholipase C, phospholipase D, and adenylyl cyclase. In some instances the last named is inactivated by a G-protein (Table 1). The products of the activated enzymes are known as second messengers (the hormone or neurotransmitter that interacts with the receptor being the first messenger). Adenylyl cyclase acts on ATP to remove two phosphates and cyclize the remaining one. The product formed is the second messenger, cyclic adenosine-3,5-monophosphate or cyclic AMP, which then combines with and activates protein kinase A. Phosphorylation of various proteins by protein kinase A brings about the appropriate cellular responses. Cyclic AMP is removed by the actions of another set of enzymes, the cyclic nucleotide phosphodiesterases. Phospholipase C acts on a particular membrane lipid to produce two second messengers, inositol trisphosphate and diacylglycerol. The former acts on intracellular receptors on the endoplasmic reticulum to cause calcium ion release which mediates a number of cellular responses. Diacylglycerol binds to, and activates, protein kinase C which also mediates a number of cellular responses

including cell growth and multiplication. Diacylglycerol is also produced under the influence of activated phospholipase D, which acts on a different membrane lipid from that which is the substrate for phospholipase C.

It will be clear from the above discussions that phosphorylation of proteins is a common biological mechanism for inducing a conformation change and that protein kinases are critical points in the amplification and distribution of signals within the cell. A single protein kinase may phosphorylate many different target proteins, some of which may themselves be additional protein kinases that then lead to further amplification in the signal cascade, until the cell responds appropriately.

Not only are there many different surface receptors and subtypes of them, but several components of the second messenger systems (G-proteins, the activated enzymes, protein kinases, phosphodiesterases, receptors for second messengers) exist in a number of isoforms. For example, protein kinase C occurs as seven isoforms which are generally tissue specific, and there are five forms of phospholipase C, six forms of adenylyl cyclase and five families of cyclic nucleotide phosphodiesterases. All isoforms are potential sites for selective drug action. For example, the five forms of phosphodiesterase can each be inhibited by a different drug,[18,19] and inhibitors of protein kinase C, which is involved in cell proliferation, are being tested as anti-cancer agents. Certain G-proteins, incidentally, are the sites of action of cholera toxin and pertussis toxin.

It would be expected that since protein phosphorylation is an important event in the signalling cascade, then cells must also possess enzymes (phosphatases) for turning off the signal by removing the phosphate group. Several protein phosphatases that fulfill this function have been characterized. In some instances, protein phosphatase activity is directly regulated by extracellular chemical messengers. Protein phosphatases too are therefore targets for selective drug action.

4. Receptors inside the cell. These are the exception rather than the rule, for most hormones and neurotransmitters do not readily penetrate cell membranes. Amongst hormones, exceptions are the steroid and the thyroid hormones. Vitamin D, which is chemically related to the steroid hormones, also acts in this way. These substances readily penetrate the membrane and interact with receptor sites in the nucleus where they act to initiate synthesis of particular proteins that then bring about the appropriate response.

A totally unrelated substance with important control functions in the body that also acts intracellularly is nitric oxide (and possibly also carbon monoxide). It came as a surprise a few years ago when it was realized that the simple molecule, nitric oxide (NO), plays a wide ranging and important role both in physiology and in pathology. Nitric oxide is formed from the amino acid L-arginine under the influence of the enzyme nitric oxide synthase, which exists in at least two forms, a constitutive and an inducible form. The freshly synthesized nitric oxide passes easily into adjacent cells, where it activates the cytosolic enzyme soluble guanylyl cyclase. This is quite a different enzyme from the membrane form that is activated by atrial natriuretic peptide although the product, cyclic GMP, is the same. The physiological effects of nitric oxide are mediated by cyclic GMP. Nitric oxide is formed in and released from endothelial cells in response to any processes, including the actions of some endogenous chemicals and some drugs, that cause elevation of Ca^{2+} and activation of nitric oxide synthase. Nitric oxide then acts on the smooth muscle of blood vessels to cause vasodilation and on the

platelets to reduce their stickiness. It is involved in maintaining the patency of blood vessels and in preventing intravascular clotting. Damage to the endothelium resulting in deficient production may therefore have serious consequences, although nitric oxide is not the only endothelium-derived substance involved; the prostaglandin called prostacyclin and an as yet unidentified hyperpolarizing factor also play a part. Excessive release of nitric oxide from the endothelium contributes to the circulatory collapse in bacteraemic shock. Nitric oxide also functions as a neurotransmitter, both in the peripheral nervous system and in the brain. In the latter case, it may have a role in learning and memory. Excess nitric oxide is cytotoxic. It may be involved in spreading neuronal damage after a stroke, and macrophages and neutrophils make use of it to kill invading bacteria.[6,20,21] The commonly used, and ancient nitrovasodilators, amyl nitrite and gylceryl trinitrate, are prodrugs, nitric oxide being released from them as one of the metabolic breakdown products.

Although the transmembrane signalling and second messenger mechanisms are hugely important, it would be incorrect to give the impression that they are the only important components of the welter of chemical signalling mechanisms that control body function, derangement of which might underly disease processes. At the level of the cell membrane, there are also the cellular adhesion molecules. These are carbohydrate and protein components of the membrane that stick cells together like a molecular Velcro®. Dysfunction in adhesion mechanisms may allow metastasising tumour cells to escape and drift around the body lodging in distant parts where they give rise to secondary tumours. Adhesion molecules also play a role in the exit of lymphocytes from the blood stream to the site of an infection. Dysfunction in, or inappropriate, cell adhesion may also play a part in inflammatory diseases (e.g. rheumatoid arthritis), in reperfusion arrhythmias of the heart, and in multiple sclerosis. The binding of infecting organisms (bacteria, viruses and protozoa) to cells involves similar adhesion mechanisms. Currently, there is therefore considerable interest in the design of drugs that can impair inappropriate cellular adhesion. In this connection, the possibilities of using oligosaccharides as templates for novel lead compounds are being explored.[22]

All of the chemicals that control the immune system are candidates for modification by drugs. Two of them, the cytokines called interleukin-1 and tumour necrosis factor, are especially relevant to leucocyte adhesion mechanisms. During infection and inflammation the affected tissues secrete cytokines which stimulate the local venules to produce certain adhesion molecules that then attract leucocytes to the site. Excessive leucocyte activity may lead to rapid and extensive tissue damage. A locally injected antibody to tumour necrosis factor has recently been found to be beneficial in rheumatoid arthritis.[23]

Numerous chemical mechanisms inside the cell, in addition to the second messenger cascades already discussed, are also potential targets for drug action. These include the whole sequence of events in gene expression, from DNA to messenger RNA and protein synthesis, including activation of the transcription factors, small DNA-binding proteins that help to determine which RNAs are made. The microtubules that form the mitotic spindle essential for cell division may be prevented from developing by drugs such as colchicine or the alkaloids from the periwinkle plant (vinblastine, vincristine), or the microtubules may be made to grow in an uncontrolled but equally devastating way by the drug taxol, obtained from the bark of the Pacific yew tree but which has now recently been

synthesized.[24] The movement of chromosomes, and any other particle, along the microtubules is controlled by the chemicals, kinesin and dynein. These chemicals possess ATPase activity and they act like outboard motors when attached to the particles, driving them, in opposite directions, along the monorails that are the microtubules.

Most of the hormones, autacoids, and neurotransmitters that act on the outer cell surface are included in Table 1, but additional targets for drug action are the multitude of specific enzymes and transport proteins involved in the synthesis and inactivation of the chemical messengers. Like the membrane receptors, enzymes generally exist in a number of isoforms so that selective drug action may be possible. An example of the importance of the existence of isozymes is the recent evidence that cyclo-oxygenase, the enzyme inhibited by aspirin, exists in at least two forms, the constitutive cyclo-oxygenase 1 and the inducible cyclo-oxygenase 2.[25] Most of the unwanted side-effects of cyclo-oxygenase inhibitors, for example on the stomach and kidney, appear to be a consequence of inhibiting cyclo-oxygenase 1, whereas their anti-inflammatory actions arise from cyclo-oxygenase 2 inhibition. Cyclo-oxygenase 2 is induced by inflammatory stimuli and by cytokines. Selective inhibitors of the latter isozyme, when they are discovered, should therefore be NSAIDs which are relatively free from side-effects.

Cyclo-oxygenase 1 is responsible for the production both of prostacyclin in the endothelium of blood vessels (which opposes thrombosis) and of thromboxane A_2 in the platelets (which favours platelet adhesion and thrombosis). Hence there is a good therapeutic case for an irreversible cyclo-oxygenase 1 inhibitor that acts selectively in the platelets, while sparing prostacyclin production. Low dose aspirin (75 mg) achieves this effect and hence is useful in the prevention of heart attacks and strokes.[26]

Just as Paul Ehrlich aimed at selective toxicity against the invading parasite rather than the host, so the modern pharmacologist extends this concept to attempt to benefit the diseased system without producing unwanted effects on otherwise normal organs. The motivation to attempt this logically lies in the expanding discoveries of subtle differences between subtypes of the multitude of enzymes, receptors, and other binding proteins that are the targets for drug action. Two final examples are given before consideration of the sources from which new drugs may be obtained. The first relates to major clinical depression and its relief. The chemical correlate of the condition seems to be a functional inadequacy of monoamine neurotransmitters in certain parts of the brain. Such neurotransmitters, after release and action at central synapses, are taken up again into the presynaptic neurone, and hence inactivated by specific transport proteins that convey the amines across the nerve terminal membrane and back into the nerve endings. The early drugs found to be effective were imipramine and close relatives, which prolong the actions of the monoamine neurotransmitters (noradrenaline and 5-hydroxytryptamine) by binding with both types of transport proteins and thereby blocking reuptake. Consequently, the transmitters persist for longer in the synaptic cleft. More recently, drugs that selectively bind to the 5-hydroxytryptamine transporter, leaving the noradrenaline transporter unaffected, have been discovered. Such drugs, which include fluoxetine, serbaline, paroxetine and fluvoxamine, are just as effective as imipramine in their anti-depressant activity, but are freer from the unwanted side-effects of the older drugs, although they may produce some different ones.[27,28]

A second example for consideration involves receptors for dopamine in the CNS. Dopamine functions as a neuro-transmitter in four main regions: the mesolimbic system, where it is involved with emotion and in which a functional excess of activity seems to be basic to schizophrenia; the nigrostriatal pathway where it is concerned with movement, and where deficiency leads to parkinsonism; the vomiting centre in which dopaminergic neurones are part of the vomiting pathway; the hypothalamic-pituitary connection where dopamine serves as the inhibitory transmitter for prolactin release. So far five dopamine receptor subtypes, D_1–D_5, have been delineated. Unfortunately, it seems that all of the functions mentioned above involve D_2 receptors. Consequently, the older dopamine antagonists useful for alleviating the symptoms of schizophrenia, inevitably, at least in high doses, may lead to a drug-induced parkinsonism, and the consequences of excessive prolactin secretion (gynaecomastia, galactorrhoea).

The D_4 receptor generally resembles the D_2 receptor in its sensitivity to anti-psychotic drugs. However, there are some pharmacological differences. For example, certain atypical drugs of this class (e.g. clozapine) have considerably more affinity for D_4 than for D_2 receptors. Clozapine retains anti-psychotic activity but is much less likely to produce drug-induced parkinsonism. This observation provides hope that more selective anti-psychotic drugs may be produced. (Unfortunately, clozapine itself occasionally causes haematological side-effects.) Recent evidence indicates that there are polymorphic forms of the human D_4 receptor that display pharmacological differences. An important question is whether the genetic tendency to schizophrenia is linked to the prevalence of a particular form of the D_4 receptor, since this would lead to the possibility of even more selective drugs (for discussion, see Iversen[29]).

It would be a mistake to suppose, and we do not intend to imply, that chronic disease persists as a consequence of a defect in a single component of a transducer mechanism. Even the simplest programmed and integrated response is controlled by multiple extracellular signals, often presented in a specific temporal sequence, and there is complex cross-talk and fine tuning between the various components of the second messenger systems. Hence, the nature of drug treatment may have to change at different stages of a disease. Even so, the change will again involve dysfunction in a chemical control system.

SOURCES OF NEW DRUGS

A truism, that is nevertheless not always appreciated, is that drugs cannot produce effects of which cells are not already capable. They can only enhance or inhibit the cells' natural functions. Since it is not unlikely that one or other abnormality of such functions is the basis of a disease, then a drug that produces the opposite action or that prevents the abnormal action is a potentially useful therapeutic agent. Hence, it is a reasonable proposition that a novel compound that produces a pronounced biological change might be at least a starter compound from which a new medicine might eventually be developed. Accordingly, the random screening of novel chemical entities, though hugely expensive, is not quite as blindly optimistic as it might appear. In large pharmaceutical companies, batteries of sensitive tests for drug action may be set up, with the use of robotic systems for the addition of compounds. Because drug action is often not predictable, serendipity, interacting with a prepared mind, still plays an important part.

Therefore, all manner of compounds are often screened, including those 'off the shelf' that have been saved after failure in some previous directed programme. With automated data collection and analysis, very large numbers of tests are feasible. Typically, several hundred thousand compounds could be tested in a year, and one of the greatest challenges is finding a sufficient number of compounds to match the screening capability. Of course this kind of approach is intellectually dissatisfying, except perhaps for the engineers who invent the robots and design the automated data collection and analysis, but because of the vast numbers of compounds randomly screened, even a very low success rate may be profitable. Naturally, however, as academic pharmacologists, we hope that the days of this kind of molecular roulette are numbered. At the present time, the cost of the original investigative programme for a new drug, including the essential toxicity tests and early clinical trials, is estimated at some tens of millions of pounds, and only about 1 in 100,000 compounds synthesized or otherwise obtained proves of real value. It is often not realized that only about 1 in 4 marketed drugs ever makes a profit equal to the costs of developing it.[30] Hence, the few outstandingly commercially successful ones, e.g. the 'ranitidines' and the 'atenolols', must make a profit during their patent life, that is big enough to support the losses made, not only by the failures, but also by the therapeutically useful nonprofit makers.

Natural products

The demand for new compounds to be tested in high throughput assays has reawakened interest in natural products as sources of novel chemicals. Despite popular opinion, 'natural' does not of course mean 'safe'; the margin of safety can in fact be terrrifyingly small, as with digitalis for example.

Amongst natural products, we must also include the polypeptide toxins from venomous animals—snakes, spiders, insects, scorpions, snails and so on.[31–34] Frequently, the purified toxins produce highly selective actions on one specific component of a biological system. For example, dendrotoxins from green mamba venom bind only to certain types of potassium channnels in neuronal membranes,[35] α-conotoxin from the marine cone snail selectively binds with muscle nicotinic receptors,[36] and so on. Not only are such selectively acting toxins highly useful as biological tools in experiments, but they may also provide starter compounds for highly selective drugs. It has not of course been the aim of evolution, in plants or animals, to produce medicine for man or for domestic animals. However, once the active molecule, or even the active component of that molecule (the so-called pharmacophore), has been delineated, then medicinal chemists can often improve upon it in terms of efficacy and freedom from side-effects. There are numerous examples from the past. Thus, synthetic local anaesthetics are improvements over cocaine, synthetic muscle relaxants over tubocurarine, synthetic anticholinesterases over physostigmine, and so on. Modern techniques can more accurately define the pharmacophores of newly studied natural products, so this type of approach remains worthy of further effort.

Rational drug design

Once the particular endogenous chemical system that is dysfunctioning in disease has been defined, then in principle a suitable type of drug to counteract that

dysfunction can be designed in the mind of the pharmacologist. After discussion with medicinal chemists, appropriate molecules for testing can be synthesized by the latter scientists. This type of approach was that of Sir James Black when he invented β-adrenoceptor blockers for counteracting certain actions of adrenaline and noradrenaline, and H_2-histamine receptor blockers for counteracting the effects of endogenous histamine on gastric acid secretion. Black's success is familiar to us all. He continues to use the same approach in other fields, as do others who follow his example. Drugs that mimic or antagonise ATP, adenosine, glutamic acid, tachykinins, cannabinoids, endothelins, angiogenins and others are being sought in these ways.

Computer-aided molecular modelling

Rational though it may be, the method referred to above is still somewhat akin to making key after key to fit a keyhole of unknown shape, and, if the drug is to be an agonist, it must not only fit the keyhole, it must also open the lock. Since the 1970s, new methods have become available for obtaining pure samples of many protein targets, as have improved methods for x-ray crystallography. At the same time, increasingly powerful and sophisticated computers, and methods for data base searching, have been developed, as have improved quantum chemical methods, molecular mechanics, molecular dynamics and Brownian dynamics. Molecular dynamics may be applied to the predicton of thermodynamic properties such as free energy differences and binding constants, and Brownian dynamics may be used to calculate effective electrostatic forces using the Poisson–Boltzmann equation, which allows the sampling of ligand binding geometrics and the prediction of the kinetics of diffusion-limited enzyme reactions. Techniques such as global energy minimization and quantum classical dynamics methods are also utilized.[37,38] These techniques may be used to obtain a three-dimensional picture of the binding site on the protein target, and to design inhibitors to fit it.[39–41] Captopril, an inhibitor of angiotensin-converting enzyme, is a now relatively primitive example of a drug that was designed by these kinds of techniques, although it depended also upon a natural product as a lead compound. Enzymes have been and remain the favoured targets, since their activity is relatively easily controlled by fitting small molecules into their catalytic sites. Nucleic acids are also possible targets for this kind of research, but drug receptors of the type listed in Table 1 and certain components of the immune system have not yet yielded to this approach. Problems include the fact that drug molecules can adopt more than one conformation and it is impossible to predict what the particular conformation might be when the drug binds to its receptor. For example, the structure of unbound cyclosporin is strikingly different once it binds to its receptor on cyclophilin A.[42] Additionally, the three dimensional structure of the receptor is rarely known and in any case is not static. Finally, many of the computational approaches have been restricted to modelling the drug molecule in a vacuum rather than in the physiologically relevant environment.

Despite the difficulties, more examples of drug discovery based on computer modelling of interactions with enzymes are beginning to appear.[43,44] The crystal structure of the HIV-1 proteinase enzyme was used to design non-peptide inhibitors, and the compound coded Ro31-8959 shows considerable promise.[45] Scientists at Agouron Pharmaceuticals have designed and synthesised inhibitors of

thymidylate synthase, and the compounds are being examined as potential anticancer agents.[46] Thymidylate synthase is the rate-limiting step in the synthesis of thymidine nucleotides, and is therefore critical for cell division. This folate-dependent pathway can be affected by the clinically-used dihydrofolate reductase inhibitor, methotrexate, but more effective and less toxic agents are still required. Appelt and colleagues describe the use of a combination of X-ray crystallography, computational chemistry, computer graphics and molecular biology in order to refine existing lead compounds and to create structurally novel lead compounds. Thymidylate synthase from the bacterium *E. coli* was used because human enzyme was not initially available. However, there is about 75 per cent homology in the protein sequences around the active sites of the enzymes from humans and *E. coli*, and a series of mutated bacterial enzymes were readily prepared in quantities sufficient for X-ray crystallographic studies. The *de novo* design of new lead compounds was based on the use of a computer program that calculated the interactions between different types of functional chemical groups and a known protein structure. Another example is that of the inhibitors of purine nucleoside phosphorylase, an enzyme of the purine salvage pathway, which is important in T-lymphocytes. Inhibitors of the enzyme may potentially be useful in T-cell leukaemias and in some autoimmune diseases, such as rheumatoid arthritis.[47] In this case, the crystal structure of the enzyme was solved, and then the effects of drug binding on the structure of enzyme-drug complexes were determined. New analogues were computer-designed, based on this structural analysis, and found to be much more potent than any previously known compounds.

Biotechnology in drug design

Biotechnology is not a separate entity from the rest of this discussion for its powerful molecular techniques are utilized in the elucidation of receptor subtypes (Table 1) and their structures, in the cloning and elucidation of all manner of binding proteins and other components of cells upon which drugs act, and in the production of assay systems (cloned receptors, transgenic animals) on which drugs may be tested. Nevertheless, it may be useful to summarize here the various contributions that molecular biology has made, and is making, to drug design and development.[48]

Genes as drugs. There has been a long cherished hope that patients afflicted by an inherited disorder might be treated by replacing their defective genes with normal genes. Obviously where a disease is the result of multiple gene defects, the problem is insurmountable in the forseeable future, although not necessarily for all time. However, more than four thousand inherited single gene disorders are known and in these cases there is considerable hope. Such single gene disorders include severe combined immune deficiency (adenosine deaminase deficiency), cystic fibrosis, Duchene muscular dystrophy, haemophilia, Huntington's disease, sickle cell anaemia, and Tay–Sachs disease. Genetic manipulations that involve the germ cells involve not only serious ethical problems (since the modification is passed on to succeeding generations), but also the fear of mutagenesis. There is less danger and less ethical concern when the manipulation is confined to the somatic cells of an already affected patient. In order to attempt gene therapy it must be possible to clone the normal human gene, and a safe method must be available for efficiently introducing the normal gene into the

cells of the patient. An important technique for delivery of the genes is to make use of an RNA-containing retrovirus. The required gene is cloned into a recombinant retrovirus vector that has been disabled so that it cannot replicate viral particles.

The first clinical trials of gene therapy for adenosine deaminase deficiency began in 1990 in the USA, and in 1993 in the UK. Normal adenosine deaminase gene was added to a child's bone marrow cells in the laboratory, and these were then reinjected. In the same year, gene therapy for cystic fibrosis commenced in the UK. The correct gene was inserted into liposomes which were sprayed into the nose. The final results are not yet known. Gene therapy is very much in its infancy, but progress is so rapid—it is only about 15 years ago since the first human gene was cloned and sequenced—that future promise is bright indeed.

Antisense and antigene oligonucleotides. The object of this technique is to interfere with the information flow from gene to protein, so that the production of the particular disease-producing protein, or viral replication, is inhibited. Thus, synthetic, 'false' oligonucleotides that bind to single- or double-stranded nucleic acids are potential candidates for therapeutic agents targetted at specific genes, either at the mRNA (antisense) or the double stranded DNA (antigene) level. Diseases (and proteins) that might be appropriate targets for this type of therapy include *Candida albicans* and *Herpes simplex* infections, asthma (against 5-lipoxygenase), rheumatoid arthritis (against phospholipase A_2), and cancer (against oncogenes). The technique works well in cultured cells, but it is not without problems when applied to human beings or intact animals.[49] An 'oligo' for use in human beings must meet at least six criteria: (1) it must be easily synthesized in bulk, (2) it must be stable *in vivo*, (3) there must be an appropriate delivery system that will convey the oligo, to its intracellular site of action, (4) it must be retained within the cell, (5) it must be able to interact with its intracellular target, and (6) it must be selective in its action and not interact with other macromolecules. Most of the problems have been overcome for some agents and several clinical trials are in progress, especially in cancer patients.

Recombinant proteins. Therapeutic proteins have captured most of the publicity for drug discovery in the last decade. Combining industrial biotechnology with molecular cloning opens the way to producing large quantities of biologically active proteins for medicinal use. The genes specifying human proteins may be transferred to bacteria (especially *E. coli*), to yeast cells or to mammalian cells in culture. Examples of human proteins or polypeptides so produced include insulin, growth hormone, relaxin, cytokines, erythropoietin, factors VIII and IX, tissue plasminogen activator, and colony stimulating factors. Additionally, other proteins used as human medicines can be produced more efficiently in this way. Hirudin, the anticoagulant from the medicinal leech, is an example. Although most of these products have fulfilled their therapeutic promise, others have been disappointing (e.g. tissue plasminogen activator) in comparison with other therapies.

Since the proteins have to be injected, some are potentially antigenic on repeated use, and all are expensive to produce. Accordingly, there is great need for the ability to produce low molecular weight, non-peptide analogues. Nevertheless, a survey that compared the success rate of biopharmaceuticals and conventional new chemical entities in clinical development in the United States concluded that biopharmaceuticals were more than twice as likely to be

successfully introduced to clinical use.[50]

Biopharmaceuticals are generally targeted at a human protein that has a known function. However, recombinant techniques can also be used to produce smaller molecules with unknown function, and these molecules can then be tested in high throughput assays. Various approaches are being applied. For example, several methods to generate libraries containing millions of small peptides have been established.[51–55] Molecular biology techniques are used to create random genetic codes for small peptides, which can be expressed on the surface of bacterial phages. Screening assays are used to detect the phages with the appropriate binding peptide, and these phages can be amplifed by infection of *E. coli*. Millions of different hexapeptides[51,53] or 15-residue peptides[52] can be prepared rapidly and screened. Preliminary experiments demonstrated the feasibility of this approach to discovering small peptides that bind with high affinity to specific monoclonal antibodies or to a model target protein, such as streptavidin; however, successful application to novel drug discovery has not yet been reported. Related approaches include the production of mixtures of large numbers of randomly generated hexapeptides in solution,[55] or pentapeptides fixed on individual beads.[56] Other approaches use a combination of solid-phase chemistry, photolabile protecting groups and photolithography to produce arrays of different molecules that can be challenged with soluble receptors in binding asssays.[54] This method is not restricted to conventional amino acid residues, but it is constrained by more conventional chemistry considerations. A novel approach to combining the flexibility of chemical synthesis with the amplifying power of genetic methods has been proposed.[57] This involves using genetic tags to mark chemical sequences, and it will be interesting to see practical examples of its use.

Transgenic animals. Human genes may be incorporated into a fertilised animal egg which then follows a normal gestation to produce a transgenic animal that expresses a particular human gene. Such transgenic animals have two important uses in drug development. They may be used to produce human therapeutic proteins in a similar way to those described above under the heading *Recombinant proteins*. It is sometimes possible to arrange for such proteins conveniently to be expressed in the milk of a transgenic cow.[58] Transgenic pigs have been created that produce human haemoglobin in large quantities.

The second important use of transgenic animals in drug development is the production of models of human diseases, which can be used both for testing new drugs for effectiveness and for toxicity. Transgenic mice have been developed that express the defective human cystic fibrosis transmembrane conductance regulator gene,[59–62] that develop atherosclerosis as a result of expressing the human apolipoprotein (a) gene,[63] or that develop mammary tumours.[64] Recently, transgenic mice have been developed that express the human gene for the amyloid precursor protein, which it is hoped, despite several previous failures, might provide a model of Alzheimer's disease.[65]

Cloned human receptor systems. The genes, or the mRNAs, that specify human receptors and, where necessary, their second messenger systems may be cloned and expressed in cells which do not normally possess similar types of receptors. Toad oocytes and yeast cells are examples which have been used. New drugs can then be tested on such relatively uncomplicated systems. Activity may be detected by modern recording techniques, including patch clamping of single receptors. Alternatively, a convenient 'reporter' system may be induced in the

cells so that receptor activation results, for example, in a readily detectable colour change.[66] By adding the new drug to a battery of different cell cultures, each with a different type or subtype of human receptors, an estimate both of potency and selectivity can be obtained. However, a note of caution is necessary. The behaviour of membrane proteins, including receptors, is strongly influenced by the nature of the surrounding lipids, and the lipids of the foreign membrane may differ from those of the normal membrane to the extent that the behaviour of the receptors is not identical to that exhibited in their natural environment.

Monoclonal antibodies (the hybridoma technique). The production of monoclonal antibodies was developed by Köhler and Milstein in Cambridge in 1975. Production is based on the immortalization and proliferation of individual antibody forming B-cells. The given antigen is injected into, say, a mouse and the antibody-producing B-cells are later isolated and fused to cultured cancer cells to produce hybridomas which are immortal. These immortalised cells divide in culture, each producing only one antibody. Isolation and propagation of the individual cells leads to a large number of cell clones, each clone containing a separate antibody, i.e. a monoclonal antibody. The technique may be extended to use immortalized human B-lymphocytes, and to clone the genes for the antibody of interest by cDNA cloning. The gene can then be introduced into an expression vector which provides high level production of the human antibody.

Monoclonal antibodies have numerous uses in protein purification and as diagnostic aids, but they also have potential use as drugs, especially in cancer therapy.[67] The aim is to obtain monoclonal antibodies directed against a specific tumour antigen, and then to couple the antibody to some kind of cytotoxin, such as ricin (from castor oil seeds) or diphtheria toxin. The monoclonal antibody will then direct the toxin selectively against the tumour cells. The problem is of course to find a tumour antigen that really is specific to the tumour, but the method holds out much promise.

THE FUTURE

There is no doubt that the age of true designer drugs is now beginning, and it is reasonable to expect substantial forward leaps not only in drug therapy of diseases for which there are already some useful approaches, but also in diseases in which progress has been slow. In the foreseeable future, it is reasonable to expect that most members of affluent societies will be enabled to maintain their full faculties and health until the time of death. But the more powerful and effective drugs become, the more damage they might do if incorrectly used. The correct use of all types of medicines is already beyond the capacity of one individual, as is the design and development of new ones. Further advances can only be achieved with intense interdisciplinary collaboration between all kinds of medical scientists and clinicians. Such collaboration is unlikely to be possible even within one organisation, so that large pharmaceutical companies will require access to a broader and broader range of fundamental science, necessitating more collaboration with smaller biotechnology-based companies and with academic centres. But, in the final analysis, controlled clinical testing of a comparable degree of sophistication will be required of those in charge of patients.

REFERENCES

1 Collier HOJ. Aspirin. *Sci Amer* 1963; **209:** 97–108.
2 Dale HH. A prospect in therapeutics. *Br Med J* 1943; **ii:** 411–6.
3 Gaddum JH. Discoveries in therapeutics. *J Pharm Pharmacol* 1954; **6:** 497–512.
4 Keele C. 100 years of progress in the drug treatment of disease. *Roy Soc Health J* 1963; **83:** 325–9.
5 Midgley JM. Drug development: from sorcery to science. *Pharm J* 1988; **241:** 358–65.
6 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; **43:** 109–42.
7 Verma A, Hirsch D, Glatt CE *et al.* Carbon monoxide: a putative neural messenger. *Science* 1993; **259:** 381–4.
8 Devane WA, Hanus L, Breuer A *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992; **258:** 1946–9.
9 Weiss R. Promising protein for Parkinson's. *Science* 1992; **260:** 1072–3.
10 Kerr JFR, Wyllie A, Currie A. Apoptosis: a basic biological phenomenon. *Br J Cancer* 1972; **26:** 239–57.
11 Martin SJ, Green DR, Cotter TG. Dicing with death: dissecting the components of the apoptosis machinery. *Trends Biochem Sci* 1994; **19:** 26–30.
12 Stenlake JB. Atracurium: a contribution to anaesthetic practice. *Pharm J* 1982; **229:** 116–20.
13 Perutz M. Protein structure. New approaches to disease and therapy. New York: Freeman 1992.
14 Lands AM, Luduena FP, Buzzo HJ. Differentiation of receptors responsive to isoproterenol. *Life Sci* (part 1) 1967; **6:** 2241–9.
15 Watson S, Girdlestone D. TiPs receptor nomenclature supplement, 5th ed. *Trends Pharmacol Sci* Suppl to vol 15 1994.
16 Bowman WC. Second messenger systems as sites of drug action. *Proc R Soc Edinb* 1992; **99B:** 1–17.
17 Zhang F, Strand A, Robbins D *et al.* Atomic structure of the MAP kinase ERK2 at 2.3 Å resolution. *Nature* 1994; **367:** 704–11.
18 Nicholson CD, Challiss RAJ, Shahid M. Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends Pharmacol Sci* 1991; **12:** 19–27.
19 Thompson WJ. Cyclic nucleotide phosphodiesterases: pharmacology, biochemistry and function. *Pharmacol Therap* 1991; **51:** 13–34.
20 Rand MJ. New perspectives in neurotransmission: nitric oxide, the mediator of nitrergic transmission. *Proc Aust Physiol Pharmacol Soc* 1992; **23:** 1–16.
21 Holden K. Nitric oxide—from exhaust fumes to medical revolution. *Pharm J* 1994; **252:** 402–7.
22 Sharon N, Lis H. Carbohydrates in cell recognition. *Sci Amer* 1993; **268:** 74–81.
23 Elliott MJ, Maina RN, Feldman M *et al.* Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis Rheum* 1993; **36:** 1681–90.
24 Nicolaou KC, Yang Z, Liu JJ. Total synthesis of taxol. *Nature* 1994; **367:** 630–4.
25 Vane JR. Towards a better aspirin. *Nature* 1994; **367:** 215–6.
26 Vane JR, Botting RM (ed). Aspirin and other salicylates. London: Chapman & Hall 1992.
27 Barondes SH. Thinking about Prozac. *Science* 1994; **263:** 1102–3.
28 Concar D. Design your own personality. *N Sci* 1994; **141:** 22–6.
29 Iversen L. Dopamine receptors: which D_4 do you have? *Nature* 1992; **358:** 109.
30 Halliday RG, Walker SR, Lumley CE. R&D philosophy and management in the world's leading pharmaceutical companies. *J Pharm Med* 1992; **2:** 139–54.
31 Harvey AL. From venom to toxin to drug. *Proc R Soc Edinb* 1992; **99B:** 55–66.
32 Harvey AL (ed). Drugs from natural products. London: Ellis Harwood 1993.
33 Blagbrough IS, Usherwood PNR. Polyamine amide toxins as pharmacological tools and pharmaceutical agents. *Proc R Soc Edinb* 1992; **99B:** 67–82.
34 Ménez A, Bontemo F, Roumestand C *et al.* 'Structural' basis for functional diversity of animal toxins. *Proc R Soc Edinb* 1992; **99B:** 83–104.
35 Harvey AL, Anderson AJ. Dendrotoxins: snake toxins that block potassium channels and facilitate neurotransmitter release. *Pharmacol Therap* 1985; **31:** 33–5.
36 Marshall IG, Harvey AL. Selective neuromuscular blocking properties of α-conotoxins *in vivo*. *Toxicon* 1990; **28:** 231–4.
37 Wendoloski JJ, Shem J, Oliva MT *et al.* Biophysical tools for structure-based drug design. *Pharmacol Ther* 1993; **60:** 169–83.

[38] Lesyng B, McCammon JA. Molecular modelling methods: basic techniques and challenging problems. *Pharmacol Ther* 1993; **60:** 149–67.
[39] Richards WG. Computer-aided drug discovery. *Proc R Soc Edinb* 1992; **99B:** 105–11.
[40] Platt E, Robson B. Case studies in automatic modelling of thrombin, alpha-lactalbumin and other proteins and implications for drug design. *Proc R Soc Edinb* 1992; **99B:** 123–36.
[41] Bugg CE, Carson WM, Montgomery JA. Drugs by design. *Sci Amer* 1993; **269:** 60–9.
[42] Weber C. The NMR structure of cyclosporin A bound to cyclophilin in aqueous solution. *Biochemistry* 1991; **30:** 6563–74.
[43] Taylor G. A rational attack on influenza. *Nature* 1993; **363:** 401–2.
[44] Peters R, McKinstry RC. Three-dimensional modeling and drug development. *Bio/Technology* 1994; **12:** 147–50.
[45] Roberts NA, Shaw S. Discovery and development of the HIV proteinase inhibitor Ro31-8959. In: Adams J, Merluzzi VJ (eds). The search for antiviral drugs. Boston: Birkhäuser 1993.
[46] Appelt K, Bacquet RG, Bartlett CA *et al.* Design of enzyme inhibitors using iterative protein crystallographic analysis. *J Med Chem* 1991; **34:** 1925–34.
[47] Ealick S, Babu YS, Bugg CE *et al.* Application of crystallographic and modelling methods in the design of purine nucleoside phosphorylase inhibitors. *Proc Nat Acad Sci USA* 1991; **88:** 11540–4.
[48] Tomlinson E. Impact of the new biologies on the medical and pharmaceutical sciences. *Pharm J* 1991; **248:** 335–44.
[49] Stein CA, Cheng Y-C. Antisense oligonucleotides as therapeutic agents—is the bullet really magical? *Science* 1994; **261:** 1004–12.
[50] Bienz-Tadmor B, Dicerbo PA, Tadmor G *et al.* Biopharmaceuticals and conventional drugs: clinical success rates. *Biotechnology* 1992; **10:** 521–5.
[51] Cwirla SE, Peters EA, Barrett RW *et al.* Peptides on phage: a vast library of peptides for identifying ligands. *Proc Nat Acad Sci USA* 1990; **87:** 6378–82.
[52] Devlin JJ, Panganiban LC, Devlin PE. Random peptide libraries: a source of specific protein binding molecules. *Science* 1990; **249:** 404–6.
[53] Scott JK, Smith GP. Searching for peptide ligands with an epitope library. *Science* 1990; **249:** 386–90.
[54] Fodor SPA, Read JL, Pirrung MC. Light-directed, spatially addressable parallel chemical synthesis. *Science* 1991; **251:** 767–73.
[55] Houghton RA, Pinilla C, Blondelle SE *et al.* Generation and use of synthetic peptide combinational libraries for basic research and drug discovery. *Nature* 1991; **354:** 84–6.
[56] Lam KS, Salmon SE, Hersh EM *et al.* A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* 1991; **354:** 82–4.
[57] Brenner S, Lerner RA. Encoded combinational chemistry. *Proc Nat Acad Sci USA* 1992; **89:** 5381–2.
[58] Smith AE. The production of pharmaceutical proteins in the milk of transgenic animals. In: Harris TJR (ed). Protein production by biotechnology. London: Elsevier 1990.
[59] Clarke LL, Grubb BR, Gabriel SE *et al.* Defective epithelial chloride transport in a gene-targeted. mouse model of cystic fibrosis. *Science* 1992; **257:** 1125–8.
[60] Snouwaert JN, Brigman KK, Latour AM *et al.* An animal model for cystic fibrosis made by gene targeting. *Science* 1992; **257:** 1083–8.
[61] Dorin JR, Dickinson P, Alton EWFW *et al.* Cystic fibrosis in the mouse by targeted insertional mutagenesis. *Nature* 1992; **359:** 211–5.
[62] Hyde SC, Gill DR, Higgins CF *et al.* Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. *Nature* 1993; **362:** 250–6.
[63] Lawn RM, Wade DP, Hammer RE *et al.* Atherogenesis in transgenic mice expressing human apolipoprotein(a). *Nature* 1992; **360:** 670–2.
[64] Muller WJ, Sinn E, Pattengale PK *et al.* Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-*neu* oncogene. *Cell* 1988; **54:** 105–15.
[65] Marx J. New lead to an Alzheimer's mouse? *Science* 1993; **261:** 1520.
[66] Hobden AN, Harris TJR. The impact of biotechnology and molecular biology on the pharmaceutical industry. *Proc R Soc Edin* 1992; **99B:** 37–45.
[67] Leon JA, Goldstein NI, Fisher PB. New approaches for the development and application of monoclonal antibodies for the diagnosis and therapy of human cancer. *Pharmacol Ther* 1994; **61:** 237–78.

Proc. R. Coll. Physicians Edinb. 1995; **25:** 25–28

GENES AND THE SKIN*

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Until recently the area of overlap between genetics and dermatology seemed confined to a few rare inherited skin disorders. However, the 'New Genetics' has touched every branch of dermatology from common conditions like acne, psoriasis and eczema to the susceptibility to certain infections, skin cancers and adverse drug reactions. Furthermore, lessons from the rare genetic defects have elucidated many aspects of normal skin biology.

CHROMOSOME LOCUS AND INDIVIDUAL DISEASES

To demonstrate the impact of molecular biology on dermatology, some of the more illuminating examples are summarised here, in the order of their chromosomal positions.

The convention for denoting the position of a gene is as follows: autosomes are numbered from 1 to 22 in descending order of size. Each has a short arm ('p') and a long arm ('q') joined at the centromere. Regions visible on a Giemsa stained preparation are numbered outwards from the centromere. Regions are subdivided into bands, and sometimes sub-bands, numbered in the same way. Thus 9q34.1 means sub-band 1 of band 4 of region 3, on the long arm of chromosome 9.

Chromosome 3 and dystrophic epidermolysis bullosa

In some families the blistering disorder dystrophic epidermolysis bullosa maps to 3p21, which is also the locus for the gene COL7AI which encodes 7.[1] These patients have defective anchoring fibrils, which are normally composed of collagen 7 and hold the epidermal basement membrane on to the dermis. The result is that the epidermis easily lifts off, forming a blister.

Chromosome 4 and piebaldism

Piebaldism is a rare dominant disorder characterised by a white forelock and large hypopigmented patches on the trunk and limbs. It has been mapped to 4q12-q21 by analogy with the mouse 'W' (white-spotted) phenotype, and by study of patients with chromosomal translocations affecting this region. In several families, piebaldism has now been shown to be due to mutations in the c-KIT oncogene at 4q12-21.[2] This gene encodes a mast cell/stem cell growth factor receptor which is probably also involved in normal melanocyte migration during embryogenesis. In these patients it seems that melanocytes migrating from the neural crest do not reach their furthest destinations on the forehead, on the anterior abdominal wall, and on the limbs.

Chromosome 9, nail-patella syndrome, tuberous sclerosis and skin tumours

Several conditions have been mapped by linkage with the ABO blood group genes at 9q34.

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