Medicinal Chemistry and Drug Discovery

Eugene Hart

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PHILOSOPHY IN MEDICINE AND DRUG

If asked about philosophy in medicine, many medical students will mention 'medical ethics'. However, philosophy is more than ethics. Philosophy differs from science in that science bases its theories wholly on established facts, whereas philosophy covers in addition to that, other areas of enquiry where no entirely satisfactory facts are available. All these endeavors are necessarily based on thinking, which itself, once verbalised is a matter of combining words in propositions. For example, the premises and conclusion of an argument are propositions. Logic itself is part of a broader framework of critical thinking, *i.e.*, '…a process, the goal of which is to make reasonable decisions about what we believe and what to do'. Reasoning is thinking structured and objectified by logic. Reasoning means directing thinking

towards reaching a conclusion. Reasons are called premises, that which the reason supports is called the 'conclusion' and the whole piece of reasoning is called an 'argument'. Correct reasoning is the practical skill of applying logical principles to particular cases. Correct reasoning fuelled by satisfactory evidence produces knowledge. Knowledge may be defined as: A clear perception of fact, truth, or duty, that familiarity which is gained by actual experience and a scope of information. Common knowledge is shared knowledge between individuals.

For example, human anatomy, allergy, coagulation or acid-base balance, belong to the realm of common knowledge in medicine. Understanding of common knowledge is one of the fundamental conditions of effective communication in medicine and anywhere else.

CONTROL OF DRUGS

The control of illicitly used drugs poses a multitude of intractable problems. We do not know precisely their effect or their mode of action in the body; we cannot do more than guess about the strength, nature, purpose of the motives that cause some people to become dependent on drugs; we have even less idea of the probable effect of widespread drug use on our society. Here, as menacingly as anywhere in the field of public policy, the penalties for wrong decisions loom.

Should one be liberal or penal? If neither, where in between? One can make out a case for the possibility of total social degeneracy and breakdown consequent on movement in either direction. In particular, we fear to make the experiment of liberality; but whether we like it or not, the experiment is being made for us. Drugs are becoming part of

the lives of people in every part of society: housewives in the Welsh valleys, school-children in Sheffield, artists in Cornwall, West Indians at High Wycombe, racing cyclists at Brentford, psychiatrists and their patients at Hendon; to all of these, drugs that were unknown to ordinary people in Britain a generation ago are becoming accepted accessories of life. Technological advance reaches inwards and it is impossible to keep it in the hands of the Establishment. Regulations and attitudes towards drugs, formed when their illicit use was confined to a small, tractable minority, are now being strained.

The first problem is: *Why* do we want to control drugs? This is a hard question. Now that freer sexual behaviour is becoming assimilated into our moral system, it seems that drug-taking is moving into its place as one of those matters that are defended or attacked with more than rational heat, a question to which considerations of logic are only marginally relevant. It is probably more useful, therefore, first to examine unconscious reasons for restricting drug use.

These are various. An obvious motive is that someone under the influence of drugs can be frightening or angering; he is hard to recognise and consequently his behaviour is unpredictable. It is not until one thinks of alcohol as a drug that one realises how subtly we have adapted to its use. The drunk is immediately recognisable by the way he stands, by the expression of his face, by his gait, by the situation in which we find him, by the way he talks, even by his smell.

We know very well how he is likely to behave and we make great allowances for actions that would otherwise be unacceptable. There are other adaptations to alcohol: it is

distinctively flavoured, so one could hardly take it in ignorance. It is diluted so that the process of getting it into the bloodstream takes time and some physical effort.

UNCERTAINTY AND PROBABILITY IN MEDICINE

Statements and conclusions in our logical discourses may be made either with certainty or with some lesser degree of probability. Essentially, there are three possible 'operating systems' of reasoning in medicine:

- Probability theory and its applications
- Chaos theory and its uses in clinical research
- Fuzzy logic and fuzzy sets theory in handling imperfect or hard to interpret data

DETERMINISM VS. UNCERTAINTY

Past generations of physicians tried to make medicine 'as scientific as possible' by adopting a deterministic paradigm of medicine. This was accomplished by sending medicine to the laboratory and operating theatres. As an example, Pasteur's postulates defined infection and a surgeon could see, touch and repair a ruptured organ. In the last century, medicine shifted towards the probabilistic paradigm of medicine. In medicine, we refer to probability more than we think.

For example, a surgical resident admits a patient with an acute abdomen and concludes his or her report not by a definitive diagnosis, but by an 'impression': peritonitis, possibly ruptured. Such a formulation can also be quantified in terms of probability.Already in the 1980s, Bryant and Norman paid attention to ways of translating natural clinical

language into probabilities. In their spirit, one might ask: What does it means that an incipient sepsis is *probable* in this patient? What does it means that signs and symptoms in this young woman are *compatible* with extra-uterine pregnancy?

These authors asked 16 clinicians to estimate the likelihood of presence of disease associated with various frequent expressions in communication of clinical findings. For example, for these 16 clinicians, a 'probable' disease or other event meant a 0.8 probability of patients having it. A 'doubtful' success of treatment meant a 0.2 probability of success of clinical intervention and a 'moderate probability' indicated a 0.5 probability according to the clinicians. This is just one example of the importance of probability in the daily life of clinicians. The management of clinical problems by different physicians and its success may reflect meanings attributed to words used to reflect probability.

CLASSICAL, THEORY OF PROBABILITY

The same surgical resident may know from experience and the litreature that from 10 patients in similar conditions and at that stage of diagnostic work-up, 4 may have a ruptured. Aside from an absolute number or frequency of such an event, *i.e.* 4, this experience may be quantified in three basic ways stemming from probability theory:

- The probability of this event is quantified from 0 to 1.0. In this case, the probability is 4/10, four in 10 or 0.4 or the surgical resident can say that he is 40 per cent sure that this patient has a ruptured pending a more detailed evaluation of this case.

Probability is a quantified expression or scale of the likelihood of an event, chance, or certainty.

- Odds are the ratio of likelihood of the occurrence and non-occurrence of an event.
- Likelihood is defined in the most general terms and very broadly as *'the probability of observed results (in a sample) given the estimates of the population parameters'* or as *'the conditional probability of observed frequencies given expected frequencies'*. However, statisticians have more precise definitions for a 'likelihood function'.

Observations in medicine are often quantified in terms of probabilities and odds, which can then be considered relative frequencies (one frequency plotted against something else, such as another frequency).

Uncertainty and working with probabilities in a formal and colloquial sense is a ubiquitous and perennial attribute of medical practice and research. The most frequent question that practitioners hear from their patients is: 'Doctor, what are my chances?', whether the question relates to the probability of developing a disease (risk), of having it (diagnosis), of being cured (effective treatment) or of having good or bad outcomes (prognosis).

An interest in games of chance led to John Graunt's application of ideas of relative frequency to plague mortality statistics in seventeenth century London. More fundamental contributions to probability theory were later made by Jakob Bernouilli and Abraham de Moivre. Panufty Lvovich Chebyshev and Francis Galton also enhanced this field. One of the basic ideas of probability theory is that under ideal

conditions a repeated experiment could result in different outcomes in different trials. Such a set of outcomes is called a sample space. Our conclusions about possible outcomes depend on initial information or rules representing conditional probabilities. For example, our understanding of the spread of AIDS depends (is conditional) on our knowledge of its agent (a virus) and its mode of transmission from one person to another. Additional probabilities are produced by events themselves, such as a diagnostic process or a clinical trial. Probability theory, events are considered *a priori* as independent. Our search for causes of disease or an effective treatment seeks to disprove that events are independent, that they occur together at random only.

The truth of any argument or proposition is uncertain. Such uncertainty can be partly, completely, or never elucidated by observational or experimental analytical research. This persisting variable degree of uncertainty led to the development of the theory and practice of decision-making in medicine as outlined later in this reading. What are the causes of this uncertainty?

- Our poor knowledge of the problem under study (etiology of essential hypertension or many cancers).
- Missing data for the complete understanding of a problem if all data are needed to solve the problem. However, 'there is never enough of data'.
- All available data are not correct because of poor measurement.
- Findings are poorly interpreted.
- Time, place and patients' demographic and clinical characteristics may vary.

- Conclusions are erroneously put into practice.
- Effectiveness of implemented decisions is not realistically determined.
- Poor distinctions are made between causes of disease cases and disease as an entity as well as between causes of disease itself (risk) and those of disease course and outcomes (prognosis).

This applies to research as well as to practice.Most of our conclusions bear a certain degree of randomness. We are not always absolutely sure that the patient has the disease we diagnose. For example, we might be uncertain about the recurrence of cancer after the surgical removal of its primary lesion. Our conclusions are more or less educated guesses, based on our experience and our knowledge of axioms (uncontested facts), causes, gauging uncertainty and uses of probability. A stochastic process is a process that incorporates some degree of randomness. It is a search for truth that also accepts some degree of uncertainty.

Those who use the technical term 'stochastic' want to circumvent the ambiguities of the term *random* (governed by chance) or *statistical significance*. The Bayes theorem, formulated in the eighteenth century, was revolutionary for its probabilistic considerations that, in medicine, are mainly concentrated in the area of diagnosis *(vide infra).* However, it also applies to other kinds of evidence. For Murphy, two important conclusions can be drawn from Bayes' views:

- In all but trivial cases, there is uncertainty. There are therefore always at least two conjectures or explanations under consideration.

- The most rational assessment of evidence is that which combines the evidence included in it so far with the current further evidence *(the posterior evidence)* to produce a *revised evidence*.

One of the other important characteristics of our observations within the framework of probability theory is our 'black or white' (binary) categorisation of events. Subjects are either male or female, young or old, healthy or sick (with a disease of interest). They are treated or not, they are exposed or not to noxious factors like carcinogens or poor diet. Hence, such characteristics and events, as well as their associations may be subjects of studies based on the probabilistic view of events.

Making clinical sense of a case means explaining its logic: why the patient exhibits certain symptoms, how this patient is similar to others, how various findings relate and the mechanism by which interventions will affect the future outcome. In groups, individual details are averaged out, but these details may be critical to the causal explanation for an individual patient. Inevitably, however, a gap exists between what we understand in a given patient and what we are able to predict: this is where probability fits in'

INTERNATIONAL CONTROL OF DRUGS

One of the first international organisations to reach within the frontiers of member states of the League of Nations and now the United Nations, is the machinery that controls the manufacture and international trade in addictive drugs. As a piece of bureaucracy, it is impressive and serves as a valuable model for other international endeavours, such as

the control of nuclear energy; as a method of preventing addiction and illicit drug use throughout the world, it is singularly ineffective.

A thorough account of its operation would be tedious. It is enough to say that in theory the member countries submit yearly estimates of their manufacture and consumption of some eighty dependence-producing drugs. An elaborate sum then shows what has gone astray; the nation responsible is invited to explain itself. The major defect of this system is that illicit manufacturers submit no estimates and until 1961 no country was obliged to report its growth of poppy. Even now many countries where much poppy is grown have not the internal organisation to make this report. In its broad effects the system of treaties, conferences and conventions has slowly driven the manufacture of heroin-the most compact opiate—back into the East, nearer to the poppy fields. But any successes in controlling addiction, such as Japan's or Formosa's before the war, are due to individual rather than collective effort.

This mechanism has only secondary effects in Britain. When the machinery was being constructed during the thirties, our domestic drug problem was negligible. We were able to accede to the most demanding treaties and were glad to, both as an example to more indulgent nations and so that we could apply the most rigourous standards in our own colonies. In particular we were anxious to repress a subversive Egyptian sub-culture that centred on hashish.

It seems, that but for the needs of empire, we would not have proscribed hashish and we would now have no cannabis problem. Since, our heroin problem is home-grown and state-

financed international control of drugs is irrelevant to our troubles. If, by some disaster of public policy, we allow a real black market to arise here, there is no reason to suppose that the United Nations Narcotics Commission will cause traffickers much difficulty in getting supplies from abroad.

BLACK MARKET

A practical and moral reason for preferring some sort of liberal drug control system, is to avoid the growth of a black market. It is no longer believed that a vigourous black market in itself makes addicts but there are a number of more serious peripheral disadvantages. For one thing it occupies a number of people in work that serves no useful social purpose and it occupies an equal number of more useful people in attempts to stop it. In fact, it is probably fair to say that in a vigourous situation of repression, the black marketeers and the police work in mutually unhelpful partnership.

The black market provides the police with addicts and peddlers to arrest, seizures of drugs and prestige; the police provide the traffickers with a captive market that cannot haggle or object, whose only price ceiling is set by their own abilities to steal, rob and whore. The black market too inad vertently helps the police combat addiction, for the drugs are sadly diluted at every stage of their journey. 'Cutting' a parcel of heroin in half only five times between grower and addict will reduce its purity to 3 per cent; its price will increase several hundred times over the journey.

Thus an ounce of heroin that can be bought in Italy for $60 will fetch $28,000 on the streets of New York —a happy commercial advantage the Mafia owes entirely to the energy

of the American Department of the Treasury. The result of this dilution is that very few addicts, perhaps one in five, actually have had enough heroin to become addicted. Although a black market situation reduces addiction and drug use, it does nothing to relieve the social damage due to the addict's way of life and it also causes him to commit a great deal of expensive crime to support his habit. Winick estimates that each addict in New York has to steal up to $90,000 worth of goods a year.5

In the summer of 1969 there were rumours that a proper black market operation was being set up in London. Powdered heroin, either imported from China or made in France, was being found in police searches and it was estimated that some 40,000 fixes (enough to supply 300 addicts) were being smuggled into Britain every month. But by May 1970 Chinese heroin had almost disappeared.

Perhaps the traffic had been suppressed by the Chinese community in Britain, who take very good care of their public relations. In any case, a black market can only develop against a system of repression: as long as addicts can get nearly enough heroin without much difficulty, there can be no solid opening for a black market in opiates. It is a heartening sign of the success of the heroin containment strategy that the black market price of methadone, the slow acting, unexciting opiate substituted in some treatments for heroin has now risen to 75p to £ 1 a grain. Two years ago it could hardly be given away.

DRUG TAKERS OVERTURN GRAVESTONES

The ordinary reader must feel that something shocking has happened; he may be less sure where to put the blame.

If the boys had been drunk, the situation is clear: they were wrong to get drunk, more wrong than to behave badly. But drugs—one is not certain. This doubt and ambivalence towards drugs—or more accurately, towards acts done under the influence of drugs, the only evidence people have of their existence—encourages drug users to assert their moral exemption, to claim that it was the drug that did the deed, not they. This in turn intensifies the alienation towards drugs and makes it more difficult to develop the social adaptations to them that we have towards alcohol.

Such rejection of the possibility of human control over drugs in turn enables society to see the drug user as incurably different and therefore as a scapegoat. This is stronger in America than here; but it is necessary to point it out as one of the consequences of penal control of drugs. Trocchi puts it well:

It's a nice tangible cause for juvenile delinquency. And it lets most people out because they're alcoholics. There's an available pool of wasted-looking bastards to stand trial as corrupters of their children. It provides the police with something to do and as junkies and potheads are relatively easy to apprehend because they have to take so many chances to get hold of their drugs, a heroic police can make spectacular arrests, lawyers can do a brisk business, judges can make speeches, the big peddlars can make fortunes, the tabloids can sell millions of copies. John Citisen can sit back and watch evil get its just deserts.

Then drug use is not simply a pharmacological affair between the user's body chemistry and an alien substance; it is the cause and effect of a sub-group of society and in

many cases the drug itself—or its visible accessories—are a part of social display. Young people in particular use drugs to demonstrate their rebellion and emancipation from their elders. However unconscious the message, society receives it loud and insulting. A passage in the *Utopiates*, describing American beatnik marihuana users who react against middle-class backgrounds by their dramatically squalid way of life, analyses one facet of society's reaction. We suggest that the police officer—or citisen—is, in fact, threatened by his quite accurate but partially unconscious understanding of what some users do mean or intend by their drug use. That threat mobilises the individual's feelings about past trauma of his own, trauma experienced at the hands of parental authorities during the difficult stages of learning order and suppressing impulse.

These individuals respond with disgust, anger, revulsion and fear and 'cleanse' themselves by the standard human ploy of making the enemy external, that is of scapegoating.... The emotionally aroused police officer who calls these users 'dirty' and hates them for their 'self-indulgence' is quite an accurate diagnostician, even though the diagnosis is in the service of his own defence system.

The problem of the rebellious, dependent young has always been to find something they can do without hurting themselves, that will still look dangerous enough to force their elders to stand and fight. For the moment drugs, particularly the soft drugs-marihuana, amphetamines—do the trick nicely. So, another motive for society's interest in drug control is that here one finds something intimately connected with juvenile revolt that can be weighed, counted,

analysed, held in the hand and sworn to in court. Something rather more satisfactory as evidence of young ingratitude than long hair and contemptuous expressions.

And finally, drugs that are thought to provide ecstasy, visions or even happiness and contentment without reference to one's material or social position, pose an obvious social threat. The cohesion of society and its control over its members depend on the inability of anyone to obtain satisfactions unless he pays for them: to pay for them he must earn and in earning he conforms to the major rules of society.

The trouble with the drug user is that he has found a way to escape from society's controls: he has no family to earn and care for, he has no self-respect, he has no home, he needs no job. Quite obviously, if more than a small minority of people lived as he does, our social system, held together by the iron chains of mutual production and consumption, would fall apart. The results would be unpleasant for those who were not drug users and so it is not surprising that those whose job it is to enforce the written and unwritten laws take a very dim view of illicit drug use. Heroin, the banisher of anxiety, is particularly dangerous, since, worry—over one's status, security, old age—is the mainspring of free enterprise capitalism. One works *oneself,* because one is dedicated and finds fulfilment in the service of society: one is not so sure about all the others.

It is probably not a bad thing that they are spurred on by their anxiety over rent and retirement, the need to buy food and status-giving consumer durables. If anyone, after an injection or a pill costing a few pennies, were able to sit back

and let the world go hang, where would we all be? On the other hand, equally dangerous drugs that support the social system by stupefying those who, like many housewives, would otherwise find it intolerable, are not seen to present any problem. Paradoxically, the conscious grounds for drug control are far less easy to set out.

We just do not know enough to separate the effects of drug use from the social conditions in which it is attractive. Thus, we can hardly use Hong Kong with its 300,000 addicts in a population of 4 millions as a model for British society if, for some reason, heroin became generally available, because the economic and social conditions are so different. Probably there normal people prefer to be stupefied rather than endure ordinary life. Similarly the reported evils of hashish in underdeveloped North African countries are probably only symptoms of poverty.

The moral question is perhaps slightly easier to attack. Here, of course, I am putting my personal views. My own feeling is that adults who know what they are doing—and no one can become a heroin addict without this knowledge—should be allowed to get on with it. I am not convinced that heroin use, in a sensible climate of opinion, is necessarily worse for the sort of person who finds it attractive than the vain attempt to live an ordinary life.

We should be humble enough to consider drug use one of the freedoms we enjoy in our society. And perhaps we forget that man is a self-optimising machine; that he is built to adapt to his training and surroundings in some way that tends to increase his chances for survival. Often his behaviour seems inconvenient, intolerable, self-destructive; this is

because we can see only the half of the situation that lies outside the organism—the equally important internal arrangements of the drug user are hidden from us. We ought to consider the idea that in the situation he finds himself, his behaviour is more for his good than not. We should hesitate to interfere unless we can make radical changes either in his internal or external environments, or both and so make drug use an option that he will prefer not to exercise.

These considerations apply particularly strongly to adolescent drug users. Addiction or habituation rather than occasional use is a symptom of something badly wrong. The fact that their personalities have not finally formed means there is some hope of altering them internally and externally enough to make drug use unnecessary; and we have an obvious duty to make this attempt. But it has to be well considered; superficial efforts may well exacerbate their problems.

PRACTICAL CONSIDERATIONS RELEVANT TO DRUG CONTROL

One solution to a drug problem is to forget it. In the West, where the objective damage due directly to the use of drugs is small, much of the ultimate harm they do is caused by the reaction of hostile social attitudes. Thus in France, although the country was the international centre of the heroin traffic in the thirties and after, although heroin factories still exist round Marseilles, there are, officially, only sixty addicts and no drug problem. One suspects that this is simply due to the French tolerance for individual aberration. In the same way we have no barbiturate 'problem', although this drug is

actually and potentially much more dangerous than heroin.

However, we are hardly likely to achieve such karmic acceptance. The opposite of acceptance is penal restriction of drug use, a policy pursued, apparently without great success, in the U.S.A., Canada, Hong Kong and other countries. In fact, this policy does actually reduce *addiction*—though unfortunately it produces more virulent side-effects. It is extremely important that we, in Britain, do not make this mistake. Not only can it cause misery, degradation, illness, squalor and early death in addicts, it is brutalising for society and because of its lack of overall success generates an extremist public attitude that makes more liberal approaches politically impossible. We have this problem in a mild way with marihuana now; the Americans, many of whom feel that the penal approach was a disastrous mistake, find it difficult now to experiment with more lenient methods.

The hard line towards addiction also tends to create and extrude intractable sub-cultures of users. This process of separation is examined by Wilkins in terms of feedback loops. Briefly, his argument is that as a minority appears it begins to establish its own recognition symbols, values, ways of behaving which distinguish it from the main culture. If its activities are antisocial, pressure from the main will tend to isolate it and to deny its members access to the ordinary rewards of society. This is met by a negative reaction in the minority culture which re-intensifies its rebellion; so the loop rolls on. This analysis alone would suggest that sub-cultures must expand explosively: experience shows this is not the case. Wilkins, having provided a force of expansion in the sub-culture, does not appear to consider the countervailing

pressures which society brings to bear on it.

There seem to be two restraining forces: society's physical power to restrict, or make difficult, the sub-culture's activities and in the ultimate resort, to kill all its members; secondly, the more immediate point that as the distance between the minor and major groups increases, so the number of new recruits abnormal enough to be willing to make the jump decreases. One sees the operation of this second control in the British Nazi Party whose members are few, but very extreme. The danger in applying pressure to the several sub-species of drug user is that we reduce the willingness of the compulsive drug user to accept our society and to accept help from it and we make communication with him almost impossible. Extreme pressure, short of annihilation, simply produces a small number of absolutely intractable deviates: drug addiction in America shows this sort of position. By relaxing social pressure one runs the risk of increasing the numbers of people affected, but one also increases their accessibility. In the end we have probably gained.

A second beneficial result is that as social pressure is reduced, so more diverse agencies can help with the problem. Extreme pressure calls for the Army, something less the police, less again and one can use doctors, social workers, psychiatrists. So many doctors in America have gone to prison for giving drugs to addicts in an attempt to establish a relationship with them that useful medical and psychiatric help is now almost unavailable to the drug user, except in the few rather isolated hospitals that deal with these cases.

Another consequence of using force to suppress drug use is an inconsistency that should be unacceptable in a civilised

society. Drug users are recognised as being ill; their illness is the cause of their drug use; therefore potential drug users are ill. The police are being used to repress symptoms of conditions that need medical attention.

THE TOOLS OF MEDICINAL CHEMISTRY

IN SILICO MODELING

To overcome the many hurdles to discovering a new drug, medicinal chemists must focus on synthesising compounds with drug-like properties. One of the first tools developed to help chemists design more drug-like molecules takes advantage of an area totally under the chemist's control—the physical properties of the compounds being designed.

These are the rules developed by Chris Lipinski, sometimes referred to as the "Rule-of-Five" (Ro5), which describe the attributes drug-like molecules generally possess that chemists should try to emulate. The Ro5 states that drug-like molecules tend to exhibit four important properties, each related to the number 5 (molecular weight,500; cLogP, a measure of lipophilicity,,5; H-bond donors,5; and H-bond acceptors,10). The Ro5 can be applied all the way from library design in the earliest stages of drug discovery to the final fine-tuning process that leads to the compound selected for development. Correlating microsomal instability and/or absorption/efflux with Ro5 properties can also provide insight about the property most important for gaining improvement in these areas. As is the case with any good model, the Ro5 is based on data, in this case from hundreds of marketed drugs. Using more specific data, models to address each of

the hurdles in the drug discovery process have been developed (for comprehensive reviews.

These include models of absorption/permeability oral bioavailability brain penetration and P450 interaction. More recently, the solution of X-ray crystal structures of the P450 enzymes 3A4 and 2D6 should enable application of structure-based drug design to help minimize interactions with these metabolic enzymes.

Models for safety issues, such as genotoxicity and HERG (human ether a-go-go related-gene) interaction (which can lead to cardiovascular side effects due to QT prolongation) are also being developed. Although this profusion of in silico models offers considerable potential for overcoming hurdles in the drug discovery process, the models are only as good as the data used to build them and often the best models are those built for a single project using data from only the compounds prepared for that specific project.

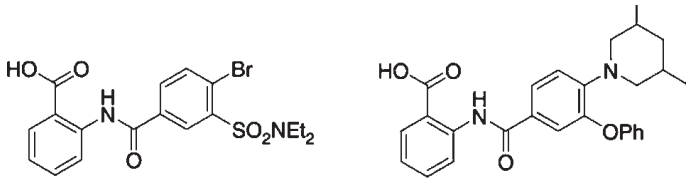
The models can be used, alone or in combination with structure-based drug design, to screen real or virtual libraries of compounds as an integral part of the design process. These improvements in library design, coupled with more efficient library synthesis and screening, provide value in both time and cost savings. The move towards using this library technology has been accelerated by the availability of a new resource for library generation: outsourcing. Contract research organisations (CROs) in the United States or offshore provide numerous synthetic services such as synthesis of litreature standards, templates and monomers for library preparation and synthesis of libraries These capabilities can relieve in-house medicinal chemists of much of the routine

synthetic chemistry so they can focus on design and synthesis to enable new structure-activity relationships (SAR) directions. For an overview of the process as it fits together for the successful discovery of new drugs.

STRUCTURE-BASED DRUG DESIGN (SBDD)

Progress in SBDD has been steady over the past two decades such that it has become a generally accepted strategy in medicinal chemistry, transforming the way medicinal chemists decide how to pursue their series' SAR. Although obtaining X-ray crystallographic data for SBDD was achieved early on, it has taken many years to learn how to interpret and not over-interpret, this data.

Structural information on the protein target provided by X-ray crystallography offers the greatest structural resolution for docking proposed ligands, but other spectroscopic techniques, such as nuclear magnetic resonance (NMR), have demonstrated their utility as well. X-ray crystallography, however, is generally restricted to analysing soluble proteins such as enzymes. Also required is a ready source of large quantities of the target protein for crystallisation, as is often the case for proteins obtained from microorganisms grown in culture. Bacterial proteins are an ideal starting point for SBDD, as in the case of the b-ketoacyl carrier protein synthase III (FabH), the target for a recent SBDD-based approach. FabH catalyses the initiation of fatty acid biosynthesis and a combination of X-ray data along with structures of substrates and known inhibitors led to selection of a screening library to provide a starting point for one recent study.



Following screening, co-crystallisation of selected inhibitors then guided the addition of functionality to take advantage of interactions with the enzyme visualised by X-ray and docking studies. A 50-fold improvement in enzyme inhibitory potency was realised in going from structure 1 to 2, accounted for by amino acid side-chain movements revealed by X-ray co-crystal structures of both compounds with the enzyme. Although much remains to be learned so that these side-chain movements can be predicted and exploited for new compound design, the study nonetheless provides a successful example of the implementation of SBDD in drug design. Although human proteins are more challenging to obtain in sufficient quantity for crystallisation, modeling based on X-ray crystal structures has been successfully applied to many human targets. Probably the best-known efforts have been in the kinase area in search of anticancer drugs, which has been reviewed recently. For example, X-ray crystallographic data revealed important aspects of the binding of the anticancer drug Gleevec (3) to its target, the Bcr-Abl kinase, including the role of the pendant piperazine group, added originally to improve solubility and the requirement for binding to an inactive conformation of the enzyme.

Combined with studies of the mutations responsible for Gleevec-resistant variants of Bcr-Abl, these studies enabled design of a new compound, BMS-354825 (4), active against

most of these resistant mutants. More recently, non-ATP binding site inhibitors have been discovered and modeled by SBDD. For example, SBDD helped to characterise a new class of p38 kinase inhibitors that bind to a previously unobserved conformation of the enzyme that is incompatible with ATP binding Insights from SBDD then guided design of a picomolar p38 kinase inhibitor based on binding to this site.

SBDD approaches to other soluble proteins have produced inhibitors of the tissue factor VIIa complex and cathepsin G. In the case of factor VIIa inhibitors, X-ray data provided information for both designing a new scaffold for inhibitors and for simultaneously improving binding affinity and selectivity over thrombin. Compound from this work was advanced to clinical trials based on its potency and selectivity for factor VIIa inhibition. The cathepsin G inhibitor programme revealed a novel binding mode for an alpha-keto phosphonate to the enzyme's oxyanion hole and active site lysine, as well as an opportunity to extend groups into a vacant binding site to improve potency. The result was a nearly 100-fold increase in inhibition following an SAR study of this direction using the amide group in compound.

Another spectroscopic technique that has been widely applied to drug design is nuclear magnetic resonance (NMR) spectroscopy (Homans, 2004). Both X-ray crystallography and NMR can be used to take advantage of the opportunity to screen fragments, small molecules with minimal enzyme affinity, but which can be linked together with structural information to form potent inhibitors.

For example, a recent approach to caspase inhibitors generated its lead structure by tethering an aspartyl moeity

to a salicylic acid group; an X-ray co-crystal structure of the most potent compound was found to mimic most of the interactions of the known peptidic caspase inhibitors. Another example explored replacement of the phosphate group found in most Src SH2 domain inhibitors with various heteroatom-containing groups by soaking fragments into a large crystal and obtaining X-ray data, leading to the 5 nM malonate-based inhibitor. For proteins that are not water soluble, such as membrane proteins, techniques that depend on crystallisation are very challenging. Homology modeling is an alternative that can be applied to transmembrane proteins such as the G-protein-coupled receptors (GPCRs), which are the target of many marketed drugs. Based on X-ray data for a prototype member of this family of proteins, bovine rhodopsin, a number of homology models for therapeutically relevant GPCRs have been built. In the case of the chemokine GPCR CCR5, a target for AIDS drugs, a homology model afforded an appreciation of the role of aromatic interactions and H-bonds involved in binding antagonists.

A three-dimensional QSAR model was next developed based on a library of potent antagonists and then combined with the homology model to confirm important interactions and indicate directions for new compound design, resulting in compound 10, a subnanomolar CCR5 antagonist. A more sophisticated approach based on docking of virtual compounds to a homology model for the neurokinin NK-1 receptor for the neurotransmitter peptide substance P has revealed structurally novel antagonists (Evers and Klebe, 2004). The most potent of these, ASN-1377642, overlaps

nicely with CP-96,345, the litreature NK-1 receptor antagonist on which the pharmacophore used for virtual screening was based. Similar combinations of SBDD-based technology are providing insights for new compound design in numerous areas of medicinal chemistry.

THE ROLE OF SYNTHETIC CHEMISTRY IN DRUG DISCOVERY

Some may ask why anything needs to be said about synthetic chemistry as a tool for drug discovery; after all, it is common to hear that "we can make anything." On the other hand, we can only carry out biological evaluation of compounds that have been synthesised. Once the evaluation of biological activity and physical properties has been used to design new targets, a suitable synthetic route must be developed. However, considerations of what can be readily prepared factor into design much earlier.

Chemists typically recognise familiar structural features for which they know a feasible synthetic route as they analyse data and properties. Design is guided by what can be readily made, especially what can be prepared as a library of compounds, so that work can begin immediately towards initiating the next round of biological testing. Although there will always be limitations to what can be synthesised based on our imperfect knowledge, recent developments in two areas have facilitated the chemist's job: analysis/purification and synthetic methodology. In the first area, routine high-field NMR instruments allow 1H-NMR and 13C-NMR characterisation of small amounts (,10 mg) of organic

compounds. Liquid-chromatography/mass spectroscopy (LCMS) and other rapid analytical techniques, combined with medium-and high-pressure chromatography, allow for ready separation of reaction mixtures. New technologies such as reactor chips and miniaturisation, supercritical fluids and ionic fluid reaction solvents and chiral separation techniques will continue to improve synthetic capabilities. In the second area, two recent advances have transformed synthetic methodology: transition-metal catalysed cross-coupling reactions and olefinmetathesis technology. The formation of carbon–carbon bonds is probably themost fundamental reaction in synthetic chemistry.

For the first several decades of the twentieth century, this reaction depended primarily on displacement of electrophilic leaving groups by enolate anions (or enamines) or addition of organometallic (*e.g.*, Grignard) reagents. The advent of palladium-catalysed coupling of more stable derivatives, such as olefins and acetylenes, boronic acids/esters and tin or zinc compounds changed this simple picture. At the same time, the development of air-stable catalysts for producing complex carbon frameworks by metathesis of olefins expanded the chemist's repertoire.

These methods allow much greater flexibility and tolerance for sensitive functional groups, enabling construction of more complicated, highly functionalised carbon frameworks. Assembling this methodology, along with that developed over the previous century, into library-enabled synthesis allows the preparation of the large numbers of compounds favoured for today's search for lead compounds using high-throughput screening (HTS) and in lead compound follow-up.

Combinatorial chemistry was initially facilitated by developments in robotic handling technology and, for solid-phase synthesis, by Merrifield peptide synthesis. Both solution-phase (Selway and Terret, 1996) and solid-phase parallel syntheses allow generation of large chemical libraries. The emphasis on these new technologies, combined with the cross-coupling and olefin metathesis synthetic methodologies, facilitates the synthesis of new classes of compounds with complex carbon frameworks.

Their emergence as lead series and the ensuing follow-up are largely the result of their preponderance in the collection of compounds screened. In other words, it can be argued that synthetic methodology creates the chemical space that is available for screening and hence, influences in a very profound way the medicines available to mankind. As the syntheses in the succeeding make clear, synthetic chemistry plays a significant role alongside medicinal chemistry in the drug discovery process.

2

Drug Discovery Process

While scientists in the ADME disciplines have historically provided support to drug discovery teams, the unique expertise, which they could bring to the discovery process, has heretofore been vastly underutilized. In no small way this may be ascribed to the less than expedient experimental procedures required to conduct most ADME studies. The time- and labour-intensiveness of much of this work is not an issue during drug development, where resources can be focused on one compound. This *modus operandi* does not conform, however, to current drug discovery efforts, which have exponentially enhanced their operating efficiency with mass-screening techniques. However, the advent of a host of exciting new technologies is providing hope that scientists in various of the ADME disciplines may now be able to participate more actively and productively in the drug discovery process.

ABSORPTION

Insofar as the overwhelming majority of new drugs are intended to be administered orally, with few exceptions the ability of an NCE to provide activity by the oral route (*i.e.* have good bioavailability) is imperative. Whereas oral bioavailability can be determined relatively easily in animal pharmacokinetic studies, it is not reasonable to expect that this approach can function optimally in a large scale-screening environment. Fortunately a number of *in vitro* and cell culture techniques have evolved in recent years that have facilitated the assessment of intestinal permeability.

It has been demonstrated that membrane permeability can be predicted for some compounds with reasonable accuracy based solely on physicochemical parameters. Therefore, close scrutiny of the chemical structure may provide valuable basic information about an NCE prior to the commencement of laboratory experiments. It is well established, for instance, that efficient oral absorption will occur only after drug has dissolved and presented itself to the intestinal mucosal surface from whence it can traverse the epithelium. Dissolution is determined by the highly interdependent influences of aqueous solubility, ionizability (pKa), and lipophilicity (octanol/water log P or log D7.4). Furthermore, log P is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility (*i.e.* increasing log P enhances permeability while reducing solubility). In light of this counterdependence, it has been suggested that oral absorption may be optimal within a log P range of 0.5 to 2.0.

While the promise of predicting membrane permeability from chemical structure alone is enticing, these methods do

not yet enjoy the level of sophistication required to supplant experimental methods. In that regard, a number of *in vitro* tools have been adapted, with notable efficiency, to high-throughput assessments of membrane permeability and potential oral bioavailability. Most notable among them are CaCO-2 cells. Derived from a human colon carcinoma cell line, these cells are grown in a confluent monolayer on porous membrane filters which are mounted in diffusion chambers. Permeability measurements are based on the rate of appearance of test compound in the receiver compartment. The apical (donor) surface of the monolayer contains microvilli and thus retains many characteristics of the intestinal brush border. The cells also express functional transport proteins and metabolic enzymes, the degree of expression being dependent upon the post-seeding age of the cells.

Everted intestinal rings and brush-border membrane vesicles (BBMV) are also commonly used systems for assessing membrane permeability. The former technique is a refinement of one of the earliest *in vitro*absorption systems in which an everted intestinal segment was suspended in a buffer system to measure mucosal-to-serosal transfer. The current methodology involves isolating a rat intestinal segment, everting, and slicing into rings, which are suspended in buffer. Removing the brush-border surface from rat or rabbit intestine and molding it into vesicles by homogenization and differential centrifugation prepare BBMV. Both everted intestinal rings and BBMV are most useful for determining drug uptake rates rather than transepithelial flux.

In the *in situ* intestinal perfusion system, an intestinal segment is exposed in an anesthetized rat and drug solution

is perfused through the lumen in a single-pass or recirculating fashion. Drug permeability is derived from the rate of disappearance of drug from the perfusate. Though more labour-intensive, in situ intestinal perfusions remain popular owing to the perceived clinical relevance of permeability data derived therefrom. In a recent study, it was demonstrated with a series of small organic molecules as well as a series of peptidomimetics that CaCO-2 cells, everted intestinal rings, and in situ perfusions have strong potential for predicting fraction absorbed in humans.

One of the most appealing attributes, which these experimental systems possess, is their capability to perform relatively high throughput screening. This is particularly relevant for CaCO-2 cells, everted intestinal rings, and BBMV. An imposing rate-limiting step, once the systems are established and optimized, is the development of assay methods (usually HPLC) to quantify the analytes of interest. On the positive side, as these experiments are conducted in aqueous buffers, the preanalytical sample purification requirement is minimal. These analytical burdens can be even further reduced by immobilized artificial membranes (IAM), a recently developed system which has been greeted with considerable enthusiasm. Briefly, the analyte is injected onto a specialized IAM HPLC column packed with a phosphati-dylcholine (PC) stationary phase selected to closely mimic biological membranes.

Theoretically, the chromatographic capacity factor for the analyte should correlate with the water-to-PC membrane partition coefficient, a useful parameter for calculating membrane permeability. The ability of IAM technology to

predict membrane permeability is currently being evaluated. Elegant in its simplicity, it is well suited to a rapid screening environment, although it is recognized that its applicability will be limited to those compounds, which are absorbed by passive processes.

METABOLISM

The systems described above have the greatest utility when absorption is rate limiting to systemic availability. For many compounds, even if absorption is optimized bioavailability may be limited by extensive metabolism. Indeed, metabolism can complicate the *in vivo* activity profile irrespective of route of administration.

As with absorption, valuable metabolic input can be imparted to the drug discovery team based on information gleaned from chemical structure alone. It is felt that the teleological underpinnings of xenobiotic transformation reside in modifying environmental toxins so as to facilitate their removal from the body. Not surprisingly, the majority of drug biotransformation processes appear to follow this paradigm, typically resulting in increasingly water-soluble metabolic products which can be more readily excreted in urine. Thus one may surmise that within a group of compounds, highly lipophilic drugs would be metabolized most actively.

More explicitly, the metabolic potential of a compound containing a chemical moiety known to be avidly biotransformed may be predicted reasonably accurately on the basis of abundant historical data. For example, being cognizant that unhindered phenolic hydroxyl groups are exquisitely good substrates for conjugating enzymes, NCEs,

which contain this moiety, can be eliminated from further consideration if a predilection to rapid metabolism is felt to be detrimental. As we gain information about the specific conformational constraints imposed by the catalytic sites of major metabolic enzymes, our ability to predict *in vivo* metabolic events based solely on chemical structure is enhanced commensurately.

Given the current state of knowledge, however, it is likely that drug discovery teams will continue to prefer metabolism-related input predicated on experimental data rather than theoretical predictions. To this end, various *in vitro* methods are available which are being increasingly incorporated into drug discovery strategies. Among the most popular and widely utilized systems in use today are hepatic microsomes. These preparations retain activity of those enzymes, which reside in the smooth endoplasmic reticulum, such ascytochromes (CYP), flavin monooxygenases, and glucuronosy ltransferases. Isolated hepatocytes appear to retain a broader spectrum of enzymatic activities, including not only reticular systems, but cytosolic and mitochondrial enzymes as well. Because of a rapid loss of hepatocyte-specific functions, it has been possible to generate useful data only with short term hepatocyte incubations or cultures. However, significant strides have been made towards maximizing the viability of hepatocytes in culture.

Liver slices, which like hepatocytes retain a wide array of enzyme activities, are also increasing in popularity. Furthermore, both hepatocytes and liver slices are capable of assessing of enzyme induction *in vitro*. The choice of which system to employ in a drug discovery screening programme

will depend on many factors, not the least of which is availability of tissue for large-scale implementation. In addition, historical information about a particular chemical series is invaluable.

Consider, for example, an SAR-driven chemical series with a prototype analogue that has been demonstrated to induce CYP activity. For metabolic screening in this programme, hepatocytes or liver slices may be preferable to microsomes. Biotransformation of NCE candidates as well as induction potential can be evaluated in the same system, making the added resources required to establish these systems well worth the investment. Information gathered from *in vitro* metabolism studies is especially useful in choosing drug candidates for future development. The potential utility of *in vitro*metabolism data in predicting *in vivo* intrinsic clearance has been touted.

Isolated heterologous human CYP enzymes have been available for several years, being expressed from cDNA in yeast (*Saccharomyces* cerevisiae), bacterial (*Escherichia coli*), and mammalian (B-lymphoblastoid) cell lines. These systems can be used to ascertain whether a compound is a substrate for a particular CYP isozyme and, if so, what metabolite is generated by that enzyme. Moreover, these enzymes, in sufficient quantity, may possibly be used as bioreactors to generate usable amounts of a metabolic product that may be difficult to chemically synthesize in the laboratory. Isozyme-specific antibodies and isozyme-specific inhibitory substrates, by selectively abolishing the activity of a particular CYP isoform, may be used to determine the relative importance of that enzyme in the turnover of an NCE.

Conversely, the ability of an NCE to interact with a particular CYP isozyme may be determined by metabolic cross-inhibition studies against prototypical CYP substrates.

In vitro metabolism systems are not limited to those derived from the liver. Most pharmaceutical companies have been building liver and tissue banks to permit a cross-comparison of metabolic turnover rates in various tissues from various species. It is not uncommon now for liver banks to house tissues from a variety of species, including those from animals treated with enzyme inducers or inhibitors. Therefore, cross-species *in vitro* metabolism comparisons are becoming more feasible and commonplace. In addition to providing information on potential rates and routes of metabolism, interspecies comparisons may help in choosing species to be used in toxicology studies.

Available *in vitro* metabolism technologies are considerably more efficient than traditional *in vivo* methods. With these systems, it is possible to assess the relative rates and routes of biotransformation of a handful of compounds in the time required to comparably characterize one compound using *in vivo* approaches. In the past, a relative paucity of tissues was the most notable impediment to conducting large-scale screening efforts with *in vitro* tools. However, the continued growth of in-house tissue banks, in concert with the optimization of isolated enzyme expression systems, has made high-throughput metabolism assessments in the discovery arena a reality.

PHARMACOKINETICS

Unfortunately, there is no substitute for actual *in vivo* data in assessing pharmacokinetic profiles of drug candidates.

While insight into various aspects of the pharmacokinetic profile (absorption, metabolism, protein binding) can be gleaned from *in vitro* techniques, there are as yet no methods available for accurately predicting what will happen to a drug when it is put into a whole animal.

For a useful assessment of pharmacokinetics and bioavailability, it is necessary to administer the drug to selected animal species both intravenously and by the intended route of administration (usually oral). Blood samples are collected over a predetermined time course after dosing and the drug is quantified in serum or plasma by a suitable bioanalytical method (*e.g.* HPLC). Alternatively or concurrently it may be possible to collect plasma samples from animals used in whole animal pharmacologic models and, based on the concentration/effect (pharmacokinetic/ pharmacodynamic) relationship established, make a link between *in vitro* pharmacologic activity and the behaviour of a compound *in vivo*.

In any case, the most significant impediment to providing pharmacokinetic input to a high-throughput discovery team is the time and labour-intensiveness of the bioanalytical methods available. To assess biochemical or pharmacologic activity *in vitro* or *in vivo*, a standardized screening method is established with a common assay endpoint that can be applied to test all compounds that are available. In contrast, a separate bioanalytical method for pharmacokinetic assessment must be developed for each compound.

For chromatographic assays, which comprise the vast majority of the methods employed in pharmacokinetic studies, remarkable improvements in assay detection limits and sample

cleanup have been realised. However, little has been done to shorten the time required for assay development and implementation. Pharmacokinetic characterization is therefore often relegated to end-stage discovery, being utilized to select which of 2 or 3 potential lead candidates has the most "acceptable" pharmacokinetic profile. Clearly, if pharmacokinetic input is to be available for early discovery decisions, more efficient methodologies are necessary. Semi-simultaneous bioavailability estimation is a screening method which has been successfully utilized in drug discovery. With this technique, an intravenous dose of drug is administered and at a suitable time postdose (*i.e.* postdistributional) an oral dose is administered to the same animal.

Pharmacokinetic parameters such as clearance and volume of distribution can be determined from the intravenous concentration-time curve. Oral pharmacokinetic parameters, including bioavilability, are subsequently extricated from the combined IV and PO concentration-time data by deconvolution. By reducing inter- and intraanimal variability, accurate pharmacokinetic data can be gathered with fewer animals. In addition, the total number of plasma samples from both routes of administration is reduced by the overlapping dosing regimes. On the other hand, a prior knowledge of the pharmacokinetic characteristics of the NCE is needed to optimize the administration paradigm. In that regard, it may be possible to extrapolate from studies with structurally analogous predecessors from the same chemical series. More critical, however, is that compound-specific analytical methods are still required for each NCE. Bioassays represent one alternative to compound-specific assays.

Plasma samples are added to an *in vitro*system and the biological activity is quantified in much the same way as a pure drug solution is tested for activity. The extent of displacement of a prototype receptor ligand or inhibition of a target enzyme activity is measured as the assay endpoint for each sample. Based on standard curves, which relate the assay endpoint to known quantities of added drug, the concentration of the compound in the plasma can be quantified. Microbiological assays, which measure the inhibition of bacterial growth on an agar plate in relation to the amount of antibacterial drug applied, are among the most widely utilized examples of this technique.

The greatest advantage of bioassays over HPLC methods is that they can be employed for analyzing any compound which possesses the appropriate biological activity, making them amenable to high throughput screening. Unfortunately, owing to their non-specific nature, these assays will quantify all biological activity in a sample, which could include active metabolites. These results may be misleading if one is attempting to understand the pharmacokinetic behaviour of only parent compound. In response, it could be argued that it is prudent to choose the compound, which provides the best activity profile, irrespective of whether the in vivo activity observed, is due to parent compound or active metabolite. Preprocessing of plasma samples (extraction) could possibly remove polar metabolites, allowing the bioassay to concentrate on parent drug.

In perhaps the most significant technologic leap in this field, scientists are capitalizing on the exquisite selectivity of mass spectrometry (MS) to circumvent the tedium of HPLC

assay development. The resulting procedure is the pharmacokinetic equivalent of a mass-screen, the simplicity and elegance of which is the result of several evolutionary steps. In its earliest incarnation, animals were dosed with a single drug and samples were collected, processed, and injected onto an HPLC- MS. The chromatographic system was optimized to isolate the drug peak and the mass spectrometer was used to detect the appropriate mass ion and quantify it. Eventually, HPLC separation was foregone in favour of direct injection of a minimally preprocessed plasma sample into the MS. In a further refinement, multiple drugs were boldly administered simultaneously to the same animal and each compound was then individually quantified.

MS is capable of distinguishing subtle differences in the characteristic mass ion peaks of each analyte, imparting a degree of specificity that HPLC cannot achieve without fairly elaborate intervention. Under this protocol, the number of drugs for which pharmacokinetic profiles can be obtained in a week increases several-fold, being limited only by the number of compounds, which are co-administered in each animal. The considerable sample burden thus engendered is easily accommodated by the facile, direct-injection MS analysis. Overcoming myriad theoretical reasons why it shouldn't work (*e.g.* potential for metabolic interactions, protein binding displacement, or additive pharmacologic effects), investigators in several large pharmaceutical companies have successfully incorporated this screening approach into routine drug discovery support.

Drug distribution and tissue penetration data are occasionally obtained during discovery screening. Plasma

protein binding assessments can be relatively easily performed using well-known and widely-used methods, providing perspective on the relationship between total *in vivo* plasma drug concentrations and those of pharmacologically available unbound drug. For more specific applications, cell cultures techniques are available for predicting the ability of compounds to traverse target biological membranes (*e.g.* blood-brain barrier, T lymphocytes). *In vivo* microdialysis has been used for assessing drug penetration into CNS and liver, but is methodologically difficult to transform into a screening tool. In addition, these methodologies typically suffer from the same bioanalytical limitations of classical pharmacokinetic profiling. It seems reasonable to hope that direct-injection MS analysis may facilitate these types of studies in the future.

ADME IN DRUG DESIGN

With continued refinement and streamlining of its underlying technologies, ADME-relateddiscovery screening can be implemented ever earlier into the discovery process. Ultimately, this will permit scientists in the ADME disciplines to play a significant role inoptimizing new drug design. Fundamentally, that role is to identify the limiting barriers to optimal *in vivo* efficacy.

Of all the attributes, which determine a drug's ultimate *in vivo* efficacy, physicochemical behaviour is perhaps the most basic. For this reason alone it deserves considerable scrutiny by the drug discovery team. Moreover, as solubility, pKa, and lipophilicity are so integrally linked with chemical structure, physicochemical properties in some respects can

be relatively predictably manipulated by chemical modifications. Historically, chemical synthetic efforts have been guided almost exclusively by SAR intended to maximize interaction with a therapeutic target (*e.g.* receptor, enzyme). The goal of ADME-guided synthesis, on the other hand, is to maximize the ability of an NCE to access the therapeutic target *in vivo*. As these synthetic directives may often be at odds, the Holy Grail for discovery teams in the future will be to achieve an optimal balance between the interests of these groups and fully integrate the valuable information which they can each impart towards guiding new drug design.

Lipophilicity is a critical determinant of a host of ADME processes. It has been suggested, for example, that compounds with log P values between 0 and 3 or 4 (*e.g.* 0.5 to 2) are the most suitable candidates for passive transcellular absorption across intestinal epithelia. As lipophilicity is increased (log P greater than 3 or 4), solubility and hence absorption progressively decline.

In contrast, more hydrophilic compounds with log P values less than 0 are likely to traverse the epithelium more slowly via paracellular channels, although it should be appreciated that molecular size is also an important determinant of transit through the narrow (5-9 Å) paracellular channels. Similarly, it has been observed that compounds with log P values greater than 0 are likely to undergo substantial renal tubular reabsorption. By itself, this effect will tend to prolong $t_{1/2}$, however the greaterlipophilicity and minimal renal excretion will also render the compound more susceptible to metabolism. Experimental determination of lipophilicity is not a trivial matter, a fact which has limited the availability

of this information in early discovery where it could be most useful. However, mathematical predictions based on a weighting of functional groups within a molecule have been performed with impressive accuracy. As confidence in the reliability of these predictions is further developed, it will become more feasible in the synthesis of new drugs to target log P values to achieve a balance between particular ADME processes. Even more so than log P, pKa is a physicochemical property that can be readily predicted, given the vast experience amassed over many years with myriad ionizable organic functional groups. The ionization state of an NCE *in vivo* can be predicted by considering its pKa relative to pH in various body compartments. It is possible to surmise, for example, that an organic acid will present to the nephron in an anionic state, rendering it potentially susceptible to active tubular secretion by the organic anion transporter. If the compound is indeed a substrate for this transporter, renal clearance may be high and elimination from the systemic circulation rapid.

Both log P and pKa are the primary determinants of compound solubility. *In vivo*, this has a decisive bearing on oral absorption, following the premise that drugs must dissolve in the GI tract to be available to cross the intestinal membrane. Given that pH progressively increases throughout the length of the gastrointestinal tract, solubility and thus absorbability of an ionizable compound will vary accordingly. Solubility, log P, and pKa are closely intertwined determinants of renal tubular reabsorption as well. Thus, whereas compounds with log P values greater than 0 may be potential candidates for tubular reabsorption, this process will only

be likely to occur if the compound is present in urine (pH 4.5 to 8) in an un-ionized state and has not exceeded its solubility limit.

Despite the importance of physicochemical properties in determining the ADME profile, they are as yet primarily rough guidelines and cannot substitute for experimentation to bear out the validity of their predicted *in vivo* effects. Experimental data generated by the discovery tools outlined above can help put the importance of physicochemical properties into the appropriate context.

Structural modifications may also be guided by more fundamental knowledge of a particular ADME process. The stability of peptidomimetic compounds to peptidases in the GI tract may be improved by various synthetic alterations, including protection of peptide bonds with steric bulk or substitution of naturally occurring L-amino acid residues with their D-isomers. The increased GI stability potentially increases bioavailability, but must be balanced against the contribution of physicochemical properties such as lipophilicity. In addition, it is important to note that although lipophilicity and GI stability may favour intestinal absorption, high molecular weight (>600 Da) of some of these compounds could render them acutely sensitive to biliary excretion, reducing absolute systemic bioavailability in spite of their good intestinal permeability.

Among the many transport proteins, which reside in the GI mucosa, the dipeptide transporter has been shown to be an important mediator of active drug absorption. In addition to di- and tripeptides, compounds from several compound classes, which are structurally similar to di- and tripeptides,

have also been shown to be substrates for this carrier, including -lactams and ACE inhibitors. Targeting synthesis to enhance the affinity of NCEs for this transporter may prove to be a fruitful approach to improving systemic bioavailability, particularly for polar compounds with inherently low membrane permeabilities.

In one such example, formation of a proline adduct improved the transcellular passage of L-methyldopa by transforming it into a substrate for the dipeptide transporter. In addition to being dipeptide-like, L-methyldopa-pro is also a substrate for intracellular prolidase which was specifically exploited to regenerate L-methyldopa after the adduct was transported across the apical surface of the enterocyte. As their substrate specificities are further delineated, other known transporters could be similarly targeted for enhancing bioavailability.

Prodrug strategies have successfully improved the oral bioavailability of numerous compounds. In many cases, this involves masking a polar group by esterification to increase lipophilicity and enhance the extent of absorption from the GI tract. After absorption, the ester is enzymatically hydrolyzed to release parent drug. Absorption has also been improved with prodrugs designed to enhance solubility by decreasing lipophilicity, targeting progroup cleavage for the GI tract. With this approach, the nature of the progroup predetermines whether parent drug is released in the GI lumen or at the brush border membrane. The ideal candidate for the latter manipulation is a highly lipophilic compound, which readily traverses the GI mucosa, but is too insoluble to produce a reasonable soluble fraction for acceptable

bioavailability. A polar progroup (*e.g.* phosphate, amino acid) is incorporated into the molecule, markedly increasing its solubility in the GI tract. The progroup is subsequently cleaved at the intestinal brush border by the targeted enzymes (*e.g.* phosphatases, aminopeptidases), which expels the lipophilic parent compound in close proximity to the intestinal surface. The low local concentration minimizes the possibility of precipitation and allows the drug to be directly absorbed. The bioavailability of several compounds has been improved with this strategy. Soft drugs provide an interesting counterpoint to prodrugs. In both cases, the administered compound is designed to undergo a predictable metabolic modification. With prodrugs, this biotransformation event rapidly converts an inactive precursor into an active drug. Conversely, a soft drug is itself the active drug, which, after eliciting a therapeutic effect, is metabolically inactivated in a predictable manner and at a controlled rate. Several approaches to rational soft drug design have been identified, including soft analogs, activated soft compounds, natural soft drugs, the active metabolite approach, and the inactive metabolite approach. In the latter case, an inactive metabolite to a known drug is first identified. This metabolite is then chemically modified to create a new drug which:

Possesses comparable pharmacologic activity to the original drug:

- Will be biotransformed to the inactive metabolite in one step and without going through toxic intermediates, and
- May possibly be subjected to further modification if transport or binding properties require optimization.

The metabolic systems typically harnessed by soft drugs are hydrolytic enzymes (*e.g.* esterases). Monoxygenases are scrupulously avoided in the belief that hydrolyses are inestimably more predictable and controllable processes. In reality, it may be difficult to integrate the soft-drug concept into novel, innovative drug discovery programmes. Its retrometabolic philosophy is based on the premise that the metabolic fate of a pharmacologically active drug is known in explicit detail. Therefore, unless the synthetic strategy is predicated on a well-established chemical series, the information required for soft-drug design will not be available. However, the basic principles of this concept might possibly be extrapolated from successful soft drugs to novel compounds with similar functional moieties.

The guidance of drug design based on more classical metabolism theory is multifaceted. As with any strategy, an ability to predict the metabolic fate of an NCE based simply on its structure would be of untold value. To that end, several computerized " Expert systems" have been developed which are designed to promulgate for any given compound a list of likely metabolic products. Unfortunately, these systems have proven to be perhaps too general in their scope, projecting an array of potential products so extensive as to be of relatively limited use. On a slightly different tack, a vast metabolite database is currently being developed which is based on metabolism studies reported in the literature over the past 90 years. This package, from Molecular Design, interfaces with ISIS databases and is thought to hold considerable promise for providing more realistic projections of metabolic routes across species.

Molecular modeling may come to fruition in the near future. Computer assisted drug design might allow the determination of NCE-receptor compatibility to proceed concurrently with an evaluation of the ability of the NCE to fit into the active site of a particular metabolic enzyme. This capacity does not presently exist on any significant scale. It is particularly frustrating with respect to the CYP family, which is the most important of Phase 1 metabolic enzyme systems. In part because CYP is a membrane-bound enzyme in close association with a reductase, it has not yet been crystallized. Therefore, an X-ray structure, required for building a useful computer model, is not available. The multiplicity of the CYP family makes the task even more formidable. Therefore, guiding synthesis towards an optimal metabolic profile is based as yet on largely empirical insight. Substantial inroads have been made in this regard, particularly with respect to 4 of the most important CYP isozymes, CYP2D6, 2C9, 2C19, and 3A4. Models of the active sites of CYP2D6 and CYP2C19 have been hypothesized based on a comparison of chemical structures of a variety of substrates, taking into consideration the strict stereo- and regioselectivity of their transformations. The high degree of selectivity inherent in their active sites make it feasible to design drugs which may be more or less likely to interact with these isozymes.

In one example, addition of a bulky para-substituent to the basic beta-blocker nucleus ultimately yielded betaxolol, which unlike its pharmacologic brethren is resistant to the action of CYP2D6. CYP3A4, on the other hand, has to date defied attempts to define its active site, being far less discriminating in its choice of substrates. Indeed, some rather

large molecules, such as cyclosporin and macrolide antibiotics are known to be turned over by CYP3A4.

As metabolism databases expand in the coming years, a better delineation of how to chemically modify NCEs will be forthcoming, permitting more predictable interactions with CYP isozymes. Some general rules are in place with regard to avoiding chemically reactive structures. Thus, compounds containing structural elements with a high risk of producing toxic products should be strictly avoided. Examples include quinones and quinonimines, aromatic methylene-dioxy groups, and aromatic nitro groups.

Enzyme induction is more difficult to predict. There is little guidance with regard to what chemical structures are known to produce this effect, with the possible exception of broad chemical groups such as barbiturates, hydantoins, di-t-butyl phenols, etc. However, even within such series, subtle changes in chemical structure can markedly and unpredictably alter enzyme induction potential . As detailed above, pharmacokinetic characterization is often relegated to end- stage discovery, at which point compound attrition has whittled the pool of potential lead compounds to a small, manageable number. Under these circumstances, the opportunity to provide meaningful feedback, which might assist in pharmacokinetically optimizing drug design, is limited. As analytical techniques become more expeditious, pharmacokineticists will play an increasingly important role in drug design. Nonetheless, it should be clearly recognized that pharmacokinetics in and of itself is not particularly meaningful. Pharmacokinetic behaviour is highly interdependent with absorption and metabolism. However,

characterizing the pharmacokinetic profile of a compound is still of utmost importance insofar as it helps put absorption, metabolism, and other data into the proper perspective. For example, *in vivo* activity may be poor despite predictions from *in vitro*absorption and metabolism studies that an NCE will be well absorbed and not be subjected to appreciable biotransformation.

The pharmacokinetic profile reveals that the drug has a short *in vivo* half-life owing to a small volume of distribution and high renal clearance, neither of which could have been predicted accurately prior to the whole animal work. In this case, an optimization of the PK profile may be guided by a realization that chemical modifications are needed which will specifically reduce renal clearance or alter plasma protein binding.

The ability to predict *a priori* the extent of plasma protein binding is limited, although fruitful attempts have been made to define the molecular binding requirements for certain sites on human serum albumin. In general, binding is likely to increase with lipophilicity; an anionic group may enhance binding to albumin even further. In contrast, cationic molecules are virtually precluded from binding to albumin, rather favouring -1-acid glycoprotein. Ultimately, despite formidable theoretical arguments to the contrary, the role of protein binding in determining *in vivo* efficacy is still not universally predictable. For example, it has been suggested that NCEs with low protein binding are the most ideal candidates for further development. Quite often this implies low lipophilicity, which could be at odds with strategies for

optimizing membrane permeability or receptor or enzyme affinity. It has also been suggested that high protein binding is not necessarily a negative attribute and in fact may help prolong the activity of some compounds with otherwise short half-lives.

3

Pharmaceutical Drug

A pharmaceutical drug, also referred to as medicine, medication or medicament, can be loosely defined as any chemical substance intended for use in the medical diagnosis, cure, treatment, or prevention of disease.

PHARMACOTHERAPY

Pharmacotherapy is a science which focuses on the use of drugs to treat disease. This branch of the sciences involves almost every branch of medicine, and integrates a wide variety of sciences such as chemistry as well. Many people around the world benefit from pharmacotherapy each year, but pharmaceutical companies create new drugs just to make a huge profit such as Watson Pharmaceuticals. Furthermore, this is the primary reason why medications are expensive and prices are driven up yearly.

TYPES OF MEDICATIONS

FOR THE GASTROINTESTINAL TRACT (DIGESTIVE SYSTEM)

Upper digestive tract: Antacids, reflux suppressants, antiflatulents, antidopaminergics, proton pump inhibitors (PPIs), H2-receptor antagonistss, cytoprotectants, prostaglandin analogues Lower digestive tract: laxatives, antispasmodics, antidiarrhoeals, bile acid sequestrants, opioid.

FOR THE CARDIOVASCULAR SYSTEM

General: β-receptor blockers ("beta blockers"), calcium channel blockers, diuretics, cardiac glycosides, antiarrhythmics, nitrate, antianginals, vasoconstrictors, vasodilators, peripheral activators Affecting blood pressure (antihypertensive drugs): ACE inhibitors, angiotensin receptor blockers, α blockers Coagulation: anticoagulants, heparin, antiplatelet drugs, fibrinolytics, anti-hemophilic factors, haemostatic drugs Atherosclerosis/cholesterol inhibitors: hypolipidaemic agents, statins.

FOR THE CENTRAL NERVOUS SYSTEM

Drugs affecting the central nervous system include: hypnotics, anaesthetics, antipsychotics, antidepressants (including tricyclic antidepressants, monoamine oxidase inhibitors, lithium salts, and selective serotonin reuptake inhibitors (SSRIs)), antiemetics, anticonvulsants/ antiepileptics, anxiolytics, barbiturates, movement disorder (*e.g.*, Parkinson's disease) drugs, stimulants (including

amphetamines), benzodiazepines, cyclopyrrolones, dopamine antagonists, antihistamines, cholinergics, anticholinergics, emetics, cannabinoids, and 5-HT (serotonin) antagonists.

FOR PAIN AND CONSCIOUSNESS (ANALGESIC DRUGS)

The main classes of painkillers are NSAIDs, opioids and various orphans such as paracetamol, tricyclic antidepressants and anticonvulsants.

FOR MUSCULO-SKELETAL DISORDERS

The main categories of drugs for musculoskeletal disorders are: NSAIDs (including COX-2 selective inhibitors), muscle relaxants, neuromuscular drugs, and anticholinesterases.

FOR THE EYE

- *General*: Adrenergic neurone blocker, astringent, ocular lubricant
- *Diagnostic*: Topical anesthetics, sympathomimetics, parasympatholytics, mydriatics, cycloplegics
- *Anti-bacterial*: Antibiotics, topical antibiotics, sulfa drugs, aminoglycosides, fluoroquinolones
- *Anti-fungal*: Imidazoles, polyenes
- *Anti-inflammatory*: NSAIDs, corticosteroids
- *Anti-allergy*: Mast cell inhibitors
- *Anti-glaucoma*: Adrenergic agonists, beta-blockers, carbonic anhydrase inhibitors/hyperosmotics, cholinergics, miotics, parasympathomimetics, prostaglandin agonists/prostaglandin inhibitors. nitroglycerin

FOR THE EAR, NOSE AND OROPHARYNX

Sympathomimetics, antihistamines, anticholinergics, NSAIDs, steroids, antiseptics, local anesthetics, antifungals, cerumenolyti

FOR THE RESPIRATORY SYSTEM

Bronchodilators, NSAIDs, anti-allergics, antitussives, mucolytics, decongestants corticosteroids, beta-receptor antagonists, anticholinergics, steroids

FOR ENDOCRINE PROBLEMS

Androgens, antiandrogens, gonadotropin, corticosteroids, human growth hormone, insulin, antidiabetics (sulfonylureas, biguanides/metformin, thiazolidinediones, insulin), thyroid hormones, antithyroid drugs, calcitonin, diphosponate, vasopressin analogues

FOR THE REPRODUCTIVE SYSTEM OR URINARY SYSTEM

Antifungal, alkalising agents, quinolones, antibiotics, cholinergics, anticholinergics, anticholinesterases, antispasmodics, 5-alpha reductase inhibitor, selective alpha-1 blockers, sildenafils, fertility medications

FOR OBSTETRICS AND GYNECOLOGY

NSAIDs, anticholinergics, haemostatic drugs, antifibrinolytics, Hormone Replacement Therapy (HRT), bone regulators, beta-receptor agonists, follicle stimulating hormone, luteinising hormone, LHRH gamolenic acid, gonadotropin release inhibitor, progestogen, dopamine agonists, oestrogen, prostaglandins, gonadorelin, clomiphene, tamoxifen, Diethylstilbestrol

FOR THE SKIN

emollients, anti-pruritics, antifungals, disinfectants, scabicides, pediculicides, tar products, vitamin A derivatives, vitamin D analogues, keratolytics, abrasives, systemic antibiotics, topical antibiotics, hormones, desloughing agents, exudate absorbents, fibrinolytics, proteolytics, sunscreens, antiperspirants, corticosteroids

FOR INFECTIONS AND INFESTATIONS

Antibiotics, antifungals, antileprotics, antituberculous drugs, antimalarials, anthelmintics, amoebicides, antivirals, antiprotozoals

FOR NUTRITION

tonics, iron preparations, electrolytes, parenteral nutritional supplements, vitamins, anti-obesity drugs, anabolic drugs, haematopoietic drugs, [[food product drug]s

FOR NEOPLASTIC DISORDERS

cytotoxic drugs, therapeutic antibodies, sex hormones, aromatase inhibitors, somatostatin inhibitors, recombinant interleukins, G-CSF, erythropoietin

FOR EUTHANASIA

An euthanaticum is used for euthanasia and physician-assisted suicide.

Euthanasia is not permitted by law in many countries, and consequently medicines will not be licensed for this use in those countries.

LEGAL CONSIDERATIONS

Medications may be divided into over-the-counter drugs (OTC) which may be available without special restrictions,

and prescription only medicine (POM), which must be prescribed by a licensed medical practitioner. The precise distinction between OTC and prescription depends on the legal jurisdiction. A third category, behind-the-counter medications (BTMs), is implemented in some jurisdictions. BTMs do not require a prescription, but must be kept in the dispensary, not visible to the public, and only be sold by a pharmacist or pharmacy technician. The International Narcotics Control Board of the United Nations imposes a world law of prohibition of certain medications. They publish a lengthy list of chemicals and plants whose trade and consumption (where applicable) is forbidden. OTC medications are sold without restriction as they are considered safe enough that most people will not hurt themselves accidentally by taking it as instructed. Many countries, such as the United Kingdom have a third category of pharmacy medicines which can only be sold in registered pharmacies, by or under the supervision of a pharmacist.

For patented medications, countries may have certain mandatory licensing programmes which compel, in certain situations, a medication's owner to contract with other agents to manufacture the drug. Such programmes may deal with the contingency of a lack of medication in the event of a serious epidemic of disease, or may be part of efforts to ensure that disease treating drugs, such as AIDS drugs, are available to countries which cannot afford the drug owner's price. In some countries, government-regulated cannabis is available by prescription.

MODERN PHARMACOLOGY

For most of the nineteenth century, drugs were not highly effective, "if all medicines in the world were thrown into the

sea, it would be all the better for mankind and all the worse for the fishes". During the First World War, developed the treating wounds with an irrigation, a germicide which helped prevent gangrene. In the inter-war period, the first anti-bacterial agents such as the sulpha antibiotics were developed. The Second World War saw the introduction of widespread and effective antimicrobial therapy with the development and mass production of penicillin antibiotics, made possible by the pressures of the war and the collaboration of British scientists with the American pharmaceutical industry.

Medicines commonly used by the late 1920s included aspirin, codeine, and morphine for pain; digitalis, nitroglycerin, and quinine for heart disorders, and insulin for diabetes. Other drugs included antitoxins, a few biological vaccines, and a few synthetic drugs. In the 1930s antibiotics emerged: first sulfa drugs, then penicillin and other antibiotics. Drugs increasingly became "the center of medical practice". In the 1950s other drugs emerged including corticosteroids for inflammation, rauwolfia alkloids as tranqulizers and antihypertensives, antihistamines for nasal allergies, xanthines for asthma, and typical antipsychotics for psychosis. As of 2008, thousands of approved drugs have been developed. Increasingly, biotechnology is used to discover biopharmaceuticals. In the 1950s new psychiatric drugs, notably the antipsychotic chlorpromazine, were designed in laboratories and slowly came into preferred use. Although often accepted as an advance in some ways, there was some opposition, due to serious adverse effects such as tardive dyskinesia. Patients often opposed psychiatry and

refused or stopped taking the drugs when not subject to psychiatric control.

Governments have been heavily involved in the development and sale of drugs. In the Elixir Sulfanilamide disaster led to the establishment of the Food and Drug Administration, and the 1938 Federal Food, Drug, and Cosmetic Act required manufacturers to file new drugs with the FDA. The 1951 Humphrey-Durham Amendment required certain drugs to be sold by prescription. In 1962 a subsequent amendment required new drugs to be tested for efficacy and safety in clinical trials. Until the 1970s, drug prices were not a major concern for doctors and patients. As more drugs became prescribed for chronic illnesses, however, costs became burdensome, and by the 1970s nearly every country required or encouraged the substitution of generic drugs for higher-priced brand names.

PHARMACEUTICALS DEVELOPMENT

Speed and productivity are mission critical in Pharmaceutical Development. Long-term survival and success in the drug development space will go to those players who figure out how to get promising new products to market quickly while simultaneously controlling development costs. Critical chain provides pieces to the solution not found in traditional project management.

FOCUS

In late-stage development, teams have literally hundreds of tasks going on in parallel. The problem is that only a few are driving the filing date. Traditional milestone-based approaches

obscure work and hide priorities. Critical chain and PPM fix that. We have seen tremendous gains in speed and productivity come from a combination of the PPM methodology with its emphasis on practicing focused work, and our software's ability to identify key tasks and resource areas.

DECISION SUPPORT

In order to file sooner with the FDA, there are hundreds of potential actions the team can take that might possibly result in acceleration. But which ones should be followed? The trick is in knowing where the leverage is—what changes are really going to make a difference. By applying PPM teams know which handful of actions is truly leveraged.

IMPROVED COMMUNICATION

When it comes to managing the project pipeline, we have found that Pharma companies often have a tremendous amount of data but little real information. This results in a lack of both vertical alignment and horizontal synchronization regarding the true status of a project and what schedule outcomes are most likely. Despite all the data, senior leadership and functional lines are often not as well informed as they need to be with respect to the true status of projects. PPM and ProChain Enterprise introduce an unprecedented level of transparency and visibility to the organization.

STRATEGIC CHALLENGES

INTRODUCTION

The pharmaceutical industry must generate novel, effective, and safe therapeutics that address unmet medical

needs at a cost that is palatable to consumers globally, in a time frame that allows effective recovery of the investments in the systems and processes necessary to generate new products, and in a manner compliant with international regulations. In the long run, return on investments in therapeutics development must pay for the full economic costs of development or the process will fail economically and cease. The seriousness of this challenge can be inferred from the steady attrition of pharmaceutical companies large and small, old and new. The economic component grows steadily each year because of increasing pricing pressures, costs, generic competition, and regulatory hurdles, along with decreasing productivity.

The scientific challenges for therapeutics developers are great in every area but particularly so in neurotherapeutics. On one level, new scientific discoveries and tools create the possibility for a golden age of therapeutics development, in which many long-term scourges of humankind may be addressed in a fundamental and powerful manner. Yet while we move from symptomatic treatments to mechanistically targeted cures, we struggle to base development on fields of knowledge that are rapidly changing and far from complete and in which our understanding of the interactions between factors is fragmentary at best. Neurotherapeutics developers face unusually high failure rates and huge attendant expenses that must be covered by the small number of therapeutics that succeed. The combination of pricing constraints and trends of increasing regulatory hurdles, falling productivity, lengthening cycle times, and falling success rates is not sustainable in the long term. Many

developers have focused on strategies to improve scientific and operational performance, such as improved collaboration with academicians and regulatory agencies, better use of technologies to explore study data and design trials, greater operational effectiveness in selecting and training quality sites, monitoring and harvesting data, producing analyses, and amortization of costs over a wider base by global registration. Although important, these approaches are not enough. Issues of discovery and clinical development strategy must also be addressed. These issues are particularly pertinent to neurotherapeutics development and even more so to the development of therapeutics for neurodegenerative disorders.

A new and better paradigm must be developed for pharma to succeed economically and medically in the long term. While incremental improvement of current approaches can be useful and necessary, a more powerful and deeply penetrating change is needed to meet the challenges the therapeutics development community faces. In the last century, the pharmaceutical industry progressed from a strategy of observation and serendipity to the current strategy of rational design, which argues that mechanistic understanding of disease leads to mechanistically targeted molecules, which works if the theory of disease etiology is correct. The conceptual weakness of this strategy is that the map is not the territory.

The systems biology underlying disease is complex, the relevant mechanisms are often multiple, interactive, and variable across species, the individual variability in an outbred human population is great, and the statistical noise

generated by imperfect clinical assessment instruments together are a huge barrier to overcome. Narrowly targeted molecular interventions based on simplistic mental maps of the disorder sometimes work but often run afoul of these complicating factors. The next strategic approach, Biodesign, potentially surpasses the performance of the rational design strategy by embracing complexity by using the capacity of the immune system to rapidly generate the myriad potential probes needed to assess the therapeutic potential of different interventions in conjunction with *in vivo*assessment technologies targeted to the human disease model and integrated with computerized disease models. Elements of this proposed paradigm include the use of disease modeling to select better targets and potential therapies, along with information-science-based approaches to enhance small molecule chemistry, exploitation of the potential for biological technologies to rapidly generate mechanistic probes, and development of different strategies for using animal models and enhanced strategies and abilities to study human molecules mechanistically and biologically.

The synergistic use of these approaches will change the overall business model of companies engaged in the development of neurotherapeutics as well as other therapeutic areas. The next-generation pharmaceutical company will be defined by intense, deep, and advanced focus on specific target diseases, working with defined populations and specialists to target disease mechanisms with ever-broadening power. The model will combine expertise in protein therapeutics and small molecules and use them interactively in devising commercially viable

therapeutics. This development will both depend on and generate a new set of relationships and interdependencies between academicians, regulators, and industry. In so doing, the Biodesign paradigm may revolutionize medicine and solve the economic challenges facing healthcare systems and therapeutics developers alike.

TERRAIN

In determining the strategy best suited to creating the next and better generation of medical therapies, it is critical begin with a map—a conceptual picture of the terrain, the challenges and obstacles—so that the most effective path forward can be plotted and followed. This essay will focus on describing key elements that must be overcome by next-generation industry strategy because pharmaceutical development sits at the intersection of two different concerns. First, there must be an unmet medical need of sufficient magnitude as to potentially justify the investments required to generate a novel therapeutic. Second, the basic science or a key observation must exist to support the hypothesis that some mechanism or molecular target or target process relevant to a disease exists and is sufficiently defined as to permit discovery approaches to operate with some confidence of a potentially successful outcome. When there is an existing and potential market and a clear scientific strategy to produce clinically meaningful benefit, industrial processes come into play.

Neurotherapeutics development exists within the larger context of general pharmaceutical development but has unique scientific and economic challenges and opportunities

that make it one of the most demanding and high-risk areas for pharma. (Note that the term pharma is meant in a broad and inclusive sense primarily to define both the pharmaceutical and biotechnology industries, although many issues are equally relevant to device therapeutics.) The economic opportunities may be great, but success rates are appallingly low, costs of development are high, and timelines for return are unusually long.

OPPORTUNITY

The pharmaceutical industry was born in the 19th century through interactions among analytical chemistry isolating active compounds from natural substances, organic chemistry becoming increasingly sophisticated in modifying those compounds and producing novel therapeutics, and the progress of experimental pharmacology.[1] Historically the industry has evolved with help from serendipity, hard work from many and singular genius from a few. The early successes of the chemotherapeutics era centered on antibiotics. In the mid-20th century, technological and conceptual advances in biochemistry triggered an explosion of therapeutics targeted towards protein receptor and enzyme-target agents. More recently, advances in biotechnology have triggered a creative burst of protein therapeutics.

Technologies for developing therapeutics have improved dramatically. X-ray crystallography and other structural approaches help define target shapes with angstrom accuracy, allowing for more rational design and, at its best, virtual chemical design. Automated combinatorial chemistry

vastly speeds the ability to generate new small molecules to test against those targets. Various types of assay chips make it possible to perform tens of thousands of screenings in an hour. The combination of a vastly increased array of targets through genomics, the ability to generate large numbers of potential ligands through combinatorial chemistry, and the ability to conduct automated high-throughput screenings through *in vitro* or cell-based assays potentially creates a situation in which the magic of large numbers may potentially be more productive of therapies than the older system, emphasizing scientific reasoning and critical discourse between chemists and biologists.

The recent description of the human genome and the rapid developments in understanding how it provides cells with instructions to produce as many as 300,000 different proteins holds the promise of allowing a fundamental understanding of the molecular logic of the life process, thereby creating myriad openings for highly targeted and possibly personalized therapeutics. The development of brain imaging technologies such as positron emission tomography (PET) and functional magnetic resonance imaging opens the door to more direct and quantitative understanding of drug effects in humans. Looking at what has been accomplished and at the ongoing stream of progressive improvements across the board, the historical vector points to a future with remarkable possibilities for addressing major human health problems in all therapeutic areas, including neurotherapeutics.

ECONOMIC CHALLENGES

ECONOMIC SUCCESS

Human behaviour is typically driven by a complex combination of competing and complementary motives. Such motives, which may include interest in science, personal experiences or concerns, economic drives for reward, and humanitarian motives, drive resource allocation and aggressiveness in pursuing goals for any given therapeutics developer. The magnitude of investment required for success is so huge that it is understood that economic motives must and—from a societal resource allocation perspective—should play a strong role.

The very fact of having sufficient resources to pursue large-scale development implies economically prudent decisions in the past to generate those resources. Rational economic investment presupposes some hope of reward that must be balanced against risks. If there is huge risk in pharmaceutical development, the real impact of successful therapeutics on human lives and national economies is also huge and therefore pharmaceutical development is potentially filled with rewards that drive the economics of investment.

Unfortunately, in therapeutics development, it is impossible to predict with any certainty whether a given effort will succeed or fail, therefore costs will be incurred for both successful and failing programmes. For the overall pharmaceutical development enterprise to continue in the long term, the income that ultimately results from the successful development efforts considered in aggregate must

compensate for the costs of and capital for both the successful and unsuccessful efforts.

The same is true for individual companies. If the revenues produced by successful products do not compensate for the fully loaded costs of development, the costs of capital, and some profit incentives, the failing enterprise will eventually become extinct. The difficulty of meeting this basic condition can be inferred from the large number of pharmaceutical companies that have failed and disappeared or been acquired. Companies may merge for reasons of synergy and/or cost reduction, but the reality is that many mergers are driven by the need of endangered or nonviable entities to find a stronger partner to secure whatever residual value their assets contain, values that would be completely lost if the company went under. Conversely, the more profitable the successful efforts are, the more resources available to explore a wider range of potential projects, including inherently riskier novel approaches. Where successful efforts are less profitable, fewer resources are available to explore novel approaches and attention tends to focus on activities that are perceived as lower risk, such as the development of therapeutics based on more proven mechanisms (often derided as "me too," but conferring some valuable marketplace choices), life-cycle management of existing products, and acquisition of products for which better marketing or combination with the existing business holds the promise of commercial reward.

ECONOMIC REWARD

The human and economic burden from CNS disease is great and drives the need for significant investments in

therapeutics development. Size estimates of the CNS market depend in part on the entities considered as neurotherapeutics, *e.g.*, neurologic, psychiatric, pain, anesthetics, or drug addiction and abuse agents. In general, sales of CNS therapeutics comprise approximately 15% of total pharmaceutical sales, approximately 30 billion worldwide. About two-thirds of these sales are for psychiatric treatments; historically, more mechanisms leading to effective products have been discovered relevant to psychiatric illness than to neurologic illnesses. Effective CNS pharmaceuticals have the potential to provide a huge benefit to patients and economies. For example, the estimated annual economic costs of anxiety disorders, depression, and schizophrenia are 47 billion, 44 billion, and 33 billion per year, respectively.

These numbers reflect the current market but may vastly understate the potential market. First, many important CNS disorders have no curative treatment at all; thus if a treatment is developed, an entirely new market comes into play. Second, other disorders have only ameliorative therapies that either have limited efficacy or are associated with significant side-effects that strongly restrict their use. More effective or better-tolerated safer therapies have the potential to dramatically increase utilization and therefore market size.

Third, markets currently served by workable but suboptimal generic therapies have the potential to grow dramatically with the introduction of more effective or safer proprietary therapeutics. In assessing the economic potential of any prospective therapeutic, the unmet medical need, the

number of potential patients who could be served by a safe and effective therapy, the seriousness of the illness, and the disability entailed must be considered (and all affect the potential benefit and hence the price of a therapy). An important concern arises from these assessments. While very well-developed markets such as the market for antidepressants, anti-epilepsy agents, or anxiolytics are well-understood and valued, the market worth for diseases in which current therapeutics have had less impact, such as stroke or dystonia, is less clearly defined. In this situation there is the potential for underestimation of market size and consequent underinvestment.

ECONOMIC RISK

A few general factors control whether development of a particular therapeutic is economically feasible. The key economic determinants of reward are the size of the market, the real competitive advantages conferred by the potential new therapeutic, its anticipated patent life ("marketing life") and potential price, and the number of competitors already occupying the market. The key economic determinants of risk are success rate, development time, and development cost. In general therapeutics development, for every 5,000 to 10,000 compounds screened, typically about 250 will enter preclinical testing; of those, five will enter clinical testing and one will win approval by the countries Food and Drug Administration (FDA). Overall, only about 11% of new active substances entering clinical development are predicted to reach the market. The success rates for neurotherapeutics, however, are far lower than average.

The relative difficulty of neurotherapeutics development is illustrated by a comparison of the chance of compounds initiated into human testing to progress to eventual marketing across therapeutic areas: anti-infectives, 33%; cardiovascular, 6%; anti-cancer, 6%; and nervous system, 1%. Of equal concern is the fact that neurotherapeutic compounds fail late in development (during phase 3 pivotal testing) far more often than other compound categories. The chance that compounds initiated into pivotal trials will subsequently progress to eventual marketing across therapeutic areas is higher, but nervous system compounds are still dramatically riskier: anti-infectives, 75%; cardiovascular, 43%; anti-cancer; 32%; and nervous system, 14%.

As a rule, pivotal programmes cost about three times as much as the combined cost of phase 1 and 2 trials. Low success rates late in development hugely accelerate the financial risk of neurotherapeutics development. Of pharmaceuticals that do win approval, only one in three will produce revenues that match or exceed development costs. The revenue stream and profits produced by one pharmaceutical will basically have to pay for the tens of thousands of antecedent compounds produced, screened, and rejected along the way.

The length of development time is a key parameter governing economic risk for the developer. Because the ability to recoup the massive investments required to develop a pharmaceutical usually drops dramatically once it goes off patent, the costs of development must be recouped while the product is on patent. Longer development times directly

reduce the number of profitable years remaining for any therapeutic. In the Countrys, it takes 10 to 15 years to move a new therapeutic from discovery through regulatory approval: around 4 to 6 years for the discovery/preclinical phase, around 4 to 6 years for the clinical phase, and 1 to 2 years for the initial regulatory approvals. In a patent life of 20 years, that leaves only 5 to 10 years to recoup the fully-loaded costs of development and capital. Median development times vary more on the basis of indication rather than target organ, *i.e.*, analgesics development is comparatively rapid but progressively longer times are required for neuroleptics, Alzheimer's disease therapies, antidepressants, and stroke treatments. The two biggest factors leading to prolonged development times are the time required to enroll enough patients for studies to have statistical significance and the treatment period needed to detect an effect.

Both factors are particularly acute in neurotherapeutics development; most therapeutics for neurodegenerative diseases tend to require relatively prolonged periods of treatment and observation to detect an effect. Additionally, the real cost of a development programme includes the costs of capital, that is, the income that could have been derived had the funds been invested in a different, lower-risk investment. Long development times raise capital costs. Analyses by the Tufts Center for the Study of Drug Development have indicated that reducing the total development time by half will reduce total costs by 29%.

Development costs are a combination of fixed and variable costs. Extensive and expensive discovery resources are needed to explore mechanisms, probe interventions

molecularly, develop therapeutic molecules and delivery mechanisms, and assess toxicology and other parameters in detail. Developmental testing of potential therapies in humans, as controlled by international regulations, principles of good clinical practices, and other constraints, involves extensive efforts to conceive development plans and protocols, conduct detailed testing in thousands of patients, capture and analyse all data in robust and reliable systems, and write extensive reports and submissions.

Cost estimates for pharmaceutical development vary widely but were estimated by the Tufts Center to be approximately 802 million in 2000. This estimate included an average out-of-pocket cost per new drug of 403 million plus costs of capital; it was further noted that the capitalized post-approval development costs raise the overall pre- and post-approval cost to 877 million. This number includes out-of-pocket preclinical and clinical expenses and costs of capital for preclinical and clinical expenditures for the expenses of both project failures and successes. These costs have consistently been driven upward by the progressive increase in the number of clinical trials: from 30 in the period from 1977 to 1980, to 68 in the period from 1994 to 1995. Similarly, the number of patients per New Drug Application (NDA) has increased from 1,576 in the period from 1977 to 1980 to 4,237 in the period from 1994 to 1995.

The 802 million figure is likely conservative. As a cross-check, one could examine the research and development (R&D) budgets of most major pharmaceutical companies, divide by the portion of the 403 million related to direct expenses rather than costs of capitalization, and get a

number of predicted compounds far higher than the average yearly number of new chemical entities registered by that company. Even if the $802 million was considered as fully-loaded costs (including costs of capital) and divided into the R&D budgets of the larger companies, the resulting number would be higher than the average number of new chemical entities registered by those companies per year. In 2000, for example, 11 major pharmaceutical companies had R&D budgets greater than $2 billion per year, yet based on current pipelines and success-rate estimates, predictions suggest launch rates averaging 1.3 new active substances per year over the past 6 years. As already noted, these numbers are of all the more concern when coupled with the realization that the R&D costs are such that only three in 10 marketed drugs produce revenues that exceed or match their development costs.

INVESTMENT AND PRODUCTIVITY

Pharmaceutical R&D investments are high and growing geometrically. In 2002, members of the Pharmaceutical Research and Manufacturers of America spent approximately 32 billion on pharmaceutical R&D, which represents about a 15-fold rise over the past 20 years and exceeds the National Institutes of Health (NIH) budget of 24 billion. NIH funding is critically important for the general advance of health sciences and should not be underestimated, but a 2001 report by the NIH indicated that when specific links to pharmaceutical developments were assessed for 47 drugs with US sales of $500 million or more per year, only four drugs had been developed in part with NIH-funded

technologies. Domestic countries pharmaceutical R&D expenditures exceed those of any other major industrial sector, even high investment sectors such as computer software and services and the electrical, electronics, and aerospace industries.

Company-financed research in products affecting the CNS and sense organs was estimated at 7.3 billion in 2001, significantly exceeding the $3.9 billion expenditure for agents acting on the cardiovascular system and roughly equal to the combined $7.4 billion expenditure on products affecting neoplasms, the endocrine system, and metabolic diseases. More than 80% of larger pharmaceutical companies developing agents to treat CNS disorders. They focus primarily on larger, more well-defined indications such as depression, schizophrenia, and multiple sclerosis, or on underserved indications in which medical need is high, such as dementias, brain tumors, or substance use disorders.

Geometric increases in R&D expenditures notwithstanding, the overall productivity of pharmaceutical research in producing new chemical entities has not increased in proportion to the investment. Over the past 20 years, pharmaceutical research has increased about 1,500% but the number of approvals of new therapeutic agents has been relatively small, rising from approximately 20 per year in the 1980s to approximately 30 per year in the 1990s and currently. This failure of productivity has been partially ameliorated by the wider markets opened by globalization and regulatory developments that facilitate more global registrations of treatments. But the pressures of productivity challenges are exacerbated by both the competition from “fast

followers" and the challenge to generate new therapeutics fast enough to replace those that go off-patent. The ability of new technologies to rapidly close discovery gaps once a promising new target for development is proven greatly facilitates the capacity of fast-follower companies to exploit the discoveries of innovator companies.

This process is reflected in the progressively reduced period of marketing exclusivity and market share enjoyed by an innovator company before competitors match the initial breakthrough process. The huge economic importance of patent expiration is driven by the fact that generic production has taken an increasing share of the countries prescription pharmaceutical market. Once the patents constraining the generic use of a compound expire, the compounds are typically produced by a manufacturer with small costs to recover ($1 million to 2 million for bioequivalence studies) and no requirement to meet the significant costs required for the development of next-generation pharmaceuticals.

In a sense, the compound passes into the patrimony of humankind, where it is sold at markedly reduced prices that do not cover the costs of further research and development. Patent expiration is a huge challenge to innovator companies, in some cases engendering crisis and the risk of economic failure.

Many major pharmaceutical companies have recognized the need to double or triple their discovery output to maintain current profitability and growth in the face of generic competition. High throughput screening has been portrayed as a major and massive source of new compounds, yet analyses suggest that actual utilization is at only 2% to 7%

of installed capacity and is not likely the rate-limiting step. Later issues such as biometabolism and compound toxicology are more important limitations to discovery output and are being managed by industrializing the screening process. Price increases are unlikely to play a significant role in compensating for the productivity challenge to the pharmaceutical industry.

Because most of the expenses associated with pharmaceuticals derive from R&D and marketing costs rather than the specific cost of goods production, prices tend to be driven by the relative benefit conferred, not the cost of unit production (although in general the cost of goods is much higher for protein therapeutics than for small molecule therapeutics and can constitute a significant portion of the total price). Price pressures are high. Healthcare expenditures are a large budget item worldwide. In 1997, healthcare costs as a percentage of gross domestic product were higher in the Countrys than in other major industrialized nations. Pharmaceutical costs are a small percentage of overall healthcare costs, about 8% in the Countrys. On average, pharmaceutical costs are similar to the average telephone bill, but are of concern because they disproportionately affect vulnerable segments of the population such as the elderly.

In most of the world, pricing is tightly controlled by governments at levels that are, in aggregate, not compatible with sustaining current worldwide pharmaceutical R&D expenditures. These prices tend to cause pharmaceutical research to shift outside the borders of the countries with lower pricing and to decrease availability of therapeutics to

patients by slowing introduction of new therapies. It can be argued that pharmaceuticals may actually decrease overall healthcare costs by reducing larger expenditure items such as hospitalization. But the key strategic implication of this pressure for developers of neurotherapeutics and other drugs is the fact that increased costs of research and development are unlikely to be covered by price increases, and therefore the need to dramatically improve productivity is inescapable.

4

Drugs Treating

CHRONIC DISEASE

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes inflammation of the joints and may cause inflammation of other tissues in the body. The immune system consists of the cells and proteins in our bodies that fight infections. An autoimmune disease occurs when our immune system doesn't recognize part of our body and attacks it as if it were an invader such as a bacteria or virus. In rheumatoid arthritis, the immune system targets synovial membrane and attacks it. The synovial membrane is secretes synovial fluid into the joint. Synovial fluid is the joint fluid that lubricates and nourishes the joint. Other tissues can also be targeted by the immune system in rheumatoid arthritis, but the synovium, or synovial membrane, is generally the primary target. When the synovial membrane

is attacked, it becomes inflamed (synovitis) and can thicken and erode. As the synovial membrane is destroyed, the synovial fluid fluid is also destroyed because it is not being secreted. The surrounding structures can also become involved leading to the joint deformities that can be seen in rheumatoid arthritis. Rheumatoid arthritis is a debilitating and common disease, affecting approximately 1% of the population. Women are affected three times as often as men. It is not clear exactly what causes or triggers the autoimmune response. Many researchers believe that a bacterial, viral, or fungal infection may trigger the autoimmune response. Other researchers believe there is a genetic role in the development of rheumatoid arthritis. Despite active research, the question of what causes rheumatoid arthritis remains a debated topic. Whatever the trigger or underlying cause, it has been shown that rheumatoid arthritis is an autoimmune disease. The course of rheumatoid arthritis is often relapsing and remitting, meaning that a person can suffer with symptoms for a prolonged period of time (perhaps months or years) and then the symptoms go away for a while (perhaps years) only to recur again at a later date. The severity and chronicity of rheumatoid arthritis varies from person to person. Most people who develop rheumatoid arthritis will do so between the ages of 20 and 60. In general, the earlier that symptoms develop, the more severe the disease will be.

DIAGNOSED

There is no single test that can diagnose rheumatoid arthritis. Instead, your physician must look at your entire history, physical examination, laboratory tests and

radiographs when making the diagnosis of rheumatoid arthritis. The first step your doctor will take when diagnosing rheumatoid arthritis is take a complete medical history from you. Your age is an important factor when considering the diagnosis. Because most people who develop rheumatoid arthritis are between the ages of 20 and 60, if you fall outside of this age range it makes the diagnosis less likely. Common symptoms from rheumatoid arthritis include morning stiffness that lasts for longer than one hour, bilateral symmetrical involvement of small joints (*e.g.* both hands), and multiple joints being involved (often 3 or more). The most common joints to be involved are the fingers, hands, wrists, and feet. Often, people with rheumatoid arthritis may also complain of general fatigue, weakness, low-grade fever without an obvious source of infection, decreased appetite, weight loss, and/or muscle pain. People with rheumatoid arthritis typically will complain of difficulty performing tasks of daily living such as writing, preparing food, grasping a cup, getting dressed, and turning a doorknob.

Occasionally, rheumatoid arthritis can affect the heart (pericarditis) in which case the person may have chest pain that is worse when taking a deep breath or when lying down. Almost any organ in the body can become affected by rheumatoid arthritis. When the blood vessels themselves are involved, a vasculitis develops that can be debilitating and dangerous. These complications are more common with long-standing chronic rheumatoid arthritis. Patients with rheumatoid arthritis will typically not have pain or swelling in the finger joint closest to the fingertip (the distal interphalangeal joint). The other joints in the hand are often

affected. The affected joints are often tender to the touch. Depending on how long the person has been having symptoms, and the severity of the rheumatoid arthritis, there may also be some deformity in the fingers, hands, wrists, and other joints. Rheumatoid nodules may develop and be felt as firm lumps beneath the skin. These occur most commonly at points of pressure under the elbow and on the fingers. Usually rheumatoid nodules do not cause pain but they can become infected or put pressure on a nerve in which case they would need to be treated.

Your doctor will likely order several tests. Blood tests will reveal an anemia (decreased hemoglobin) in 80% of patients with rheumatoid arthritis. The erythrocyte sedimentation rate (ESR) is a general marker of inflammation and is elevated in 90% of patients. In 70% of rheumatoid arthritis a marker called rheumatoid factor (RF) will be present. However, all of these tests are nonspecific and may be positive in people without rheumatoid arthritis. The results of these tests need to be integrated by your physician with your other findings to make the diagnosis of rheumatoid arthritis. On radiographs (x-ray), the typical findings of rheumatoid arthritis include cysts, osteopenia, swelling, bony erosions, narrowed joint space, deformities, and fractures. Your physician may perform an arthrocentesis of the affected joint or joints. This procedure involves putting a needle under sterile conditions into the affected joint and aspirating the fluid. The fluid is then sent for analysis. This procedure is used in this setting to rule out other possible etiologies of the pain and swelling such as infection, gout, and pseudogout.

In a person with suspected rheumatoid arthritis and neck pain or neurologic symptoms (pain, numbness, weakness radiating into the arms or legs), the physician should perform tests to ensure that there is no instability of the cervical spine (vertebrae in the neck) because people with rheumatoid arthritis are more susceptible to this type of instability than the general population.

TREATMENT

There is no cure for rheumatoid arthritis. However, several treatments exist to decrease the symptoms of rheumatoid arthritis and greatly improve quality of life.

- *Early diagnosis*: The sooner that rheumatoid arthritis is treated, the better. If you think you might have rheumatoid arthritis, or other type of arthritis, don't delay in getting to your doctor. Prolonging treatment only makes it more difficult to treat later.
- *Diet*: One of the simplest treatments, and yet a treatment that has been found to be effective for many people, is consuming an anti-inflammatory diet. Primarily, this consists of consuming omega-3 fatty acid containing foods, including small cold water fish, fruits, and vegetables. See the section on diet and nutrition for more details.
- *Weight loss*: As with other forms of arthritis, if you are overweight then losing weight can make a large impact on decreasing pain and stiffness in your joints by taking some of the pressure off of them.
- *Exercise and rest*: Rest is an important part of treating rheumatoid arthritis. One of the symptoms

of rheumatoid arthritis can be fatigue as well as a sense of generalized malaise. It is important to get adequate rest so that your body does not get run down, This is true for everyone, but particularly true for people with rheumatoid arthritis. People with rheumatoid arthritis should not exercise to the point of exhaustion. Rather, they should take frequent breaks and rest before they get too tired.

While rest is important, it is also important to stay active and participate in an exercise regimen. The exercise regimen should include cardiovascular exercise (bicycling, swimming, etc), stretching, and strengthening exercises. Your physician can provide you with a structured exercise programme tailored to your individual needs. Ideally, the exercise programme should include exercises such as swimming that do not load the joints with too much pressure. It is very important to promote flexibility in the joints and move each joint through its full range of motion at least once a day.

- *Physical therapy*: It is important to be enrolled in a well-structured physical therapy programme. In addition, physicians and physical therapists can fit you for gait aids (*e.g.* cane) when necessary.
- *Occupational therapy*: Because rheumatoid arthritis affects the small joints of the wrist, hand, and fingers, as well as other joints, it can make activities of daily living difficult. Occupational therapists specialize in helping patients overcome these difficulties and perform their activities of daily living. A structured programme with an occupational therapist can include learning proper body

biomechanics, developing strategies to perform activities despite limitations, the use of splints to aid in tasks (*e.g.* hand splints, wrist splints), and other ways to use healthier body parts to compensate for body parts that are more affected by the disease process. Splints are also helpful in maintaining proper joint alignment.

- *Modalities*: Physicians, chiropractors, physical therapists, and occupational therapists may use a variety of modalities to help reduce symptoms. These modalities include ice, heat, massage, ultrasound, warm wax, and electrical stimulation.
- *Oral medications*: There are two basic lines of oral medication treatment for rheumatoid arthritis. First-line treatment is used for acute inflammation and pain. These medications include non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen, and etodolac. The most common side effect from this class of medication is abdominal problems such as stomach pain, ulcers, and bleeding in the gastrointestinal tract. Combining these medications with stomach protecting medicines like sucralfate and misoprostol can reduce the risk of stomach problems. A protonpump inhibitor medication such as omeprazole or pantoprazole (*e.g.* Prevacid, Protonix), can also be helpful to reduce the risk of stomach problems.

Steroids are also considered a first line treatment for rheumatoid arthritis. Steroids can be taken orally or injected directly into an affected joint. Long-term corticosteroid

treatment carries a significant number of risks including easy bruising, cataracts, increased risk of infection, skin necrosis, osteoporosis and muscle wasting. The risk of osteoporosis can be decreased by taking supplements of calcium and vitamin D. The risk of increased infection rates can be reduced by gradually tapering off steroids instead of abruptly stopping taking high-dose steroids (which can also result in an acute flare up of the rheumatoid arthritis).

Second-line medications include disease-modifying anti-rheumatic drugs (DMARDs). These medications are not anti-inflmmatory medications but they do promote remission of the disease. DMARDs are taken chronically for months or years. They generally take longer to be effective than first-line drugs. Depending on the response of the individual, more than one DMARD may be used at a time. While reserved as second-line therapy, many physicians believe that the sooner DMARDs are used, the better the response.

DMARDs include hydroxycholoquine, sulfasalzine, methotrexate, D-penicillamine, gold salts, azathioprine, and cyclophosphamide. Plaquenil is an antimalarial medication that has been found to be helpful. Patients taking plaquenil should be monitored by an opthamologist because there is a small but increased risk of developing vision changes. Other side effects include skin rashes, muscle weakness, stomach problems. Sulfasalzine is a medication that is typically used for inflammatory bowel disease (*e.g.* ulcerative colitis, Crone's disease). It has been found to be useful for rheumatoid arthritis. Skin rash, headache, nausea, fever, and stomach problems are the most common side effects. People with sulfa allergy should not take sulfasalzine. People with

gastrointestinal or genitourinary obstruction should also not take this drug. Methotrexate is an immunosuppressive drug that has also been shown to effective in rheumatoid arthritis. It is better tolerated than some of the other DMARDs but side effects include stomach problems, malaise, fatigue, fever, increased infection rate, and chills. Because of the small risk of bone marrow or liver dysfunction, all patients on this drug require regular blood testing. Azathioprine, cyclophosphamide, and other immunosuppressive drugs are generally reserved for severe, recalcitrant cases of rheumatoid arthritis because of the potential for serious side effects. D-penicillamine is also used to treat rheumatoid arthritis. Side effects include fever, chills, mouth sores, skin rash, and kidney and bone marrow damage. Gold salts used to be used more frequently than at present. The large number of side effects of gold salts have made them less attractive than other DMARDs. Nevertheless, in select patients they may be helpful.

Anti-TNF alpha factor medications have recently emerged as a newer class of medication for the treatment of rheumatoid arthritis. In many patients, this class of drug has been extremely helpful. Etanercept, infliximab, and adalimurnab are all members of this class of drug. TNF alpha is a potent inflammatory mediator. TNF alpha is released from damaged cells and essentially acts like a beacon calling other inflammatory cells to come to the site. Anti-TNF alpha medications stop TNF alpha from calling to other inflammatory cells and so they help break the inflammatory cycle. Etanercept is injected under the skin either once or twice a week. Infliximab is given intravenously, and

adalimumab is injected weekly or every other week. Anti-TNF alpha medications may cause stomach problems, nausea, vomiting, upper respiratory infection symptoms, and skin rash. Anti-TNF alpha medications are typically reserved for patients who have not responded to DMARD therapy. Once anti-TNF alpha therapy is begun, DMARDs are also often used in conjunction.

Injections: Local injections of cortisone are often effective in treating symptomatic joints. The bursae that cover and protect joints are also more commonly inflamed in rheumatoid arthritis. These bursae also can be injected with cortisone to reduce the inflammation and pain. Research is currently underway at academic institutions into injecting anti-TNF alpha medication directly into the affected joints. However, this is currently an investigational treatment.

Surgery: When joints become very symptomatic, interfere with activities of daily living and quality of life, and when these joints do not respond to more conservative treatments, surgical intervention can be considered. Depending on the joint and degree of involvement, multiple surgical options are available. A thorough discussion of all the potential risks and benefits should be pursued with your doctor. Total hip replacement and total knee replacement are considered when the hip and knee are involved, respectively.

GOUT

Gout, a painful and potentially debilitating form of arthritis, has afflicted such famed figures. Today it affects roughly two million Americans. This disorder develops after tiny, needle-like crystals of uric acid (a biological waste

product) accumulate in joints, causing swelling and extreme sensitivity, sometimes to the point where even the slight touch of a sheet is unbearable. The same crystals may cause kidney stones if they accumulate in the kidneys.

Gout usually affects one joint at a time, most often the big toe, but sometimes a knee, ankle, wrist, foot, or finger. If gout persists for many years, uric acid crystals may collect in the joints or tendons and under the skin, forming whitish deposits known as tophi. About 90 per cent of people with gout are men older than 40, and African American men are twice as likely as Caucasian men to be affected. Gout tends not to occur in women until at least 10 years after menopause.

CAUSES

For many people, gout develops after a combination of factors contributes to the buildup of excessive levels of uric acid in the body. Abnormally high levels of uric acid may result from a diet that is rich in purines, chemicals that are broken down into uric acid by the body. Purines can be found in anchovies, nuts, and organ foods such as liver, kidney, and sweetbreads. Sometimes, for unknown reasons, the body will produce too much uric acid regardless of diet. Gout can also develop when the kidneys excrete too little uric acid, which can happen in people with some types of kidney disease and in those who drink too much alcohol. In addition, obesity or sudden weight gain can cause elevated levels of uric acid. Some medications, particularly diuretics, also contribute to high uric acid levels. People at risk for developing gout include those with a family history of the

disease and those with hypertension, hyperlipidemia, or diabetes. Symptoms of gout pain and swelling within a joint, especially the big toe often, an initial episode that occurs at night shiny red or purple skin around the affected joint extreme tenderness around the joint.

DIAGNOSING

To reach a diagnosis, your doctor will ask you about your diet, your medication use, your alcohol consumption, and whether you have a family history of gout. During a physical exam, your doctor will inspect your inflamed joints and look for tophi on your skin. Your doctor may also use a needle to withdraw a small fluid sample from your affected joint. This fluid will be examined under a microscope to determine whether uric acid crystals are present. Your doctor may also order a blood test to determine your uric acid level, but this test is not definitive because — for a variety of different reasons — many people without gout have an elevated uric acid level, and even in people with gout, the results may be normal.

TREATMENT

Gout is usually treated with a two-prong medication strategy: The first goal is to ease attacks of joint pain and inflammation, while the second, longer-term goal is to decrease blood uric acid level and prevent further attacks.

Usually a doctor begins by prescribing a nonsteroidal anti-inflammatory drug (NSAID) to control pain and inflammation. Aspirin may raise your uric acid level; for this reason aspirin (and aspirin containing NSAIDs such as salsalate) is not a good NSAID choice to treat an attack of

gout. However, many people take low dose aspirin to reduce the risk of heart attack, stroke or other serious health problems. If you've been instructed to take low dose aspirin, don't stop taking it without discussing it with your doctor. If you cannot tolerate an NSAID or if these drugs are ineffective, your doctor may suggest a corticosteroid. Less often, high dose oral colchicine is prescribed, but be aware that this drug tends to cause unpleasant side effects (nausea, vomiting, cramps, diarrhea) and is not well tolerated in about 80 per cent of people.

For people with attacks that respond poorly to therapy, involve multiple joints, or occur frequently, or when kidney stones or tophi are present, a second type of drug may be prescribed to prevent future gout attacks. It's important to keep taking this drug even after you feel better. The first choice is usually allopurinol (Aloprim, Zyloprim), which decreases your body's production of uric acid. Other options include probenecid (Benemid) and sulfinpyrazone (Anturane), which help the kidneys to eliminate uric acid. An investigational medication, febuxostat, is not yet approved by the FDA, but has shown promise as a potential new treatment for gout. You can help prevent further attacks by avoiding diuretics, limiting your alcohol intake, drinking plenty of water, and maintaining a healthy weight. You may also want to reduce your consumption of foods that seem to trigger gout attacks, such as meat and certain types of seafood and vegetables — although many people find that strict dietary restrictions are of limited benefit.

PSEUDOGOUT

Pseudogout is a form of arthritis that occurs when a particular type of calcium crystal accumulates in the joints. As more of these crystals are deposited in the affected joint, they can cause a reaction that leads to severe pain and swelling. The swelling can be either short-term or long-term and occurs most frequently in the knee, although it can also affect the wrist, shoulder, ankle, elbow, or hand. The pain caused by pseudogout is sometimes so excruciating that it can incapacitate someone for days.

As its name suggests, the symptoms of pseudogout are similar to those of gout (see "Gout"). Pseudogout can also resemble osteoarthritis or rheumatoid arthritis. A correct diagnosis is vital, as untreated pseudogout can lead to joint degeneration and osteoarthritis. Pseudogout is most common in the elderly, occurring in about 3% of people in their 60s and as many as half of people in their 90s.

CAUSES

The cause of this condition is unknown. Because risk increases significantly with age, it is possible that the physical and chemical changes that accompany aging increase susceptibility to pseudogout. Certain medical conditions also make people more susceptible to pseudogout. These include an underactive thyroid (hypothyroidism), a genetic disorder of iron overload (hemochromatosis), or excessive blood levels of calcium (hypercalcemia). Pseudogout also can be triggered by joint injury, such as joint surgery or a sprain, or the stress of a medical illness. If the underlying condition causing pseudogout can be identified and treated,

it may be possible to prevent future attacks. Frequently, however, there is no identifiable trigger.

Symptoms of pseudogout pain, swelling, and stiffness around a single joint, especially the knee or wrist occasionally, more then one joint affected at a time fever, usually low-grade.

DIAGNOSIS

It may be difficult to diagnose pseudogout because it shares so many symptoms with gout, infection, and other causes of joint inflammation. In fact, pseudogout often occurs in people with other joint problems, such as osteoarthritis. Therefore, even when pseudogout is correctly identified, it is important to investigate whether there are other conditions present as well. Doctor may order an x-ray of the inflamed joint in order to look for calcium deposits in the cartilage, although these deposits are sometimes present in healthy elderly people who do not experience the swelling that characterizes pseudogout. To verify the presence of calcium crystals, your doctor may remove a small amount of fluid from the affected joint. This is done with a needle, after applying a numbing medication to the joint. This joint fluid is then analysed for evidence of calcium crystals, inflammation, or infection. Your doctor may also order tests for other conditions that can trigger pseudogout, including tests of calcium and thyroid function.

TREATING PSEUDOGOUT

To combat joint pain and swelling, your doctor may prescribe NSAIDs such as indomethacin and naproxen, or may give you glucocorticoid injections to keep the swelling

down (see "Corticosteroid injections"). Your doctor may also remove fluid from the inflamed joint, a procedure called aspiration, as this may help to ease the pressure and inflammation.

The combination of joint aspiration and medication usually eliminates symptoms within a few days, although the doctor may also recommend treatment with oral corticosteroids over a short period of time. Daily use of a low-dose NSAID or colchicine, a medicine that is also used in the treatment of gout, may help to prevent further attacks. Unfortunately, there is no treatment available that can dissolve the calcium crystal deposits, although the joint degeneration that often goes along with pseudogout may be slowed by treatments that decrease joint swelling. Occasionally, people with recurrent or chronic pseudogout may develop osteoarthritis. In this case, surgery (such as joint replacement) may be the only effective treatment.

ANKYLOSING SPONDYLITIS

Ankylosing spondylitis is a chronic, systemic inflammatory disease that may strike in the prime of life, often between the ages of 20 and 40. It's more common in men than in women. The disease develops as tendons attaching muscles to the spine become inflamed, causing pain and limiting movement. As ankylosing spondylitis progresses, vertebrae in the spinal column may fuse. In its most advanced stages, the disease may affect joints in the lower back and upper buttocks and also cause inflammation or damage to the eyes, heart, and lungs.

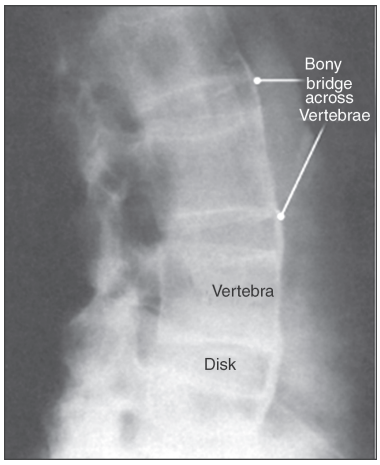


Fig. X-ray of the Spine

This x-ray shows a fused bamboo-like spine characteristic of ankylosing spondylitis.

CAUSES OF ANKYLOSING SPONDYLITIS

Ankylosing spondylitis runs in some families. An unusually high percentage of people with ankylosing spondylitis — 96% in one study — carry the HLA-B27 gene, which occurs more commonly in white people than in other racial groups. A person who carries the HLA-B27 gene has only about a 1%–2% chance of developing ankylosing spondylitis. If a parent or sibling has the condition, however, experts estimate that the risk for a person with the gene rises to 10%–20%. Conversely, not having the gene is no guarantee of protection.

DIAGNOSING ANKYLOSING

Ankylosing spondylitis is one of the more difficult rheumatic diseases to diagnose early because the symptoms are similar to other causes of low back pain. It may take up to five years after the onset of symptoms for ankylosing spondylitis to show up on an x-ray. At first, x-rays will show

that the margins of the sacroiliac joints appear indistinct. Later, the bones ankylose (or fuse).

TREATING ANKYLOSING SPONDYLITIS

Most people with ankylosing spondylitis can lead normal lives by using a combination of anti-inflammatory drugs and physical therapy. Your doctor may start by prescribing an NSAID such as indomethacin, but if this doesn't reduce the inflammation, a second choice is often a DMARD such as sulfasalazine or methotrexate. Several studies have demonstrated that anti-TNF agents are beneficial for ankylosing spondylitis. As they are FDA-approved for this condition, doctors are increasingly recommending anti-TNF drugs as a first choice of therapy. If you develop ankylosing spondylitis, you can take steps to prevent spine deformity; in fact, such measures are an essential part of treatment. At least twice a day, try to practice stretching exercises that extend the spine, preferably after a hot shower has reduced stiffness. Rheumatologists recommend swimming as the best overall exercise because it does not stress the back as much as running or other weight-bearing exercises.

DRUGS AFFECTING BLOOD PRESSURE

AIM OF TREATMENT

- The usual target is to reduce blood pressure to 140/90 or below.
- In some cases, the target is to get it below 130/80 mmHg. For example, if you have a cardiovascular disease such as a stroke or heart disease, if you have certain kidney diseases, and for some people with diabetes.

DRUG USE

There are five main classes of drugs that are used to lower blood pressure. There are various types and brands of drug in each class. The following gives a brief overview of each of the classes. However, for detailed information about your own medication you should read the leaflet that comes inside the drug packet.

ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORS

These drugs work by reducing the amount of a chemical that you make in your bloodstream called angiotensin II. This chemical tends to constrict (narrow) blood vessels. Therefore, less of this chemical causes the blood vessels to relax and widen, and so the pressure of blood within the blood vessels is reduced. There are various types and brands of ACE inhibitors. For example, captopril, cilazapril, enalapril, fisinopril, lisinopril, perindopril, quinapril, ramipril, and trandolapril. An ACE inhibitor is particularly useful if you also have heart failure or diabetes. ACE inhibitors should not be taken by people with certain types of kidney problems, people with some types of artery problems, and if you are pregnant. You will need a blood test before starting an ACE inhibitor to check that your kidneys are working well. The blood test is repeated within two weeks after starting the drug, and within two weeks after any increase in dose. Then, a yearly blood test is usual.

ANGIOTENSIN RECEPTOR BLOCKERS

These drugs are sometimes called angiotension II receptor antagonists. There are various types and brands. For

example, candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan and valsartan. They work by blocking the effect of angiotensin II on the blood vessel walls. So, they have a similar effect to ACE inhibitors (described above).

CALCIUM-CHANNEL BLOCKERS

These drugs affect the way calcium is used in the blood vessels and heart muscle. This has a relaxing effect on the blood vessels. Again, there are various types and brands. For example, amlodipine, diltiazem, felodipine, isradipine, lacidipine, lercanidipine, nicardipine, nifedipine, nisoldipine, and verapamil. Calcium-channel blockers can also be used to treat angina.

DIURETICS ('WATER TABLETS')

The most commonly used diuretic to treat high blood pressure in the UK is called bendroflumethiazide - but there are others. For example, chlorothiazide, chlorthalidone, cyclopenthiazide, hydrochlorothiazide, and indapamide. Diuretics work by increasing the amount of salt and fluid that you pass out in your urine. This has some effect on reducing the fluid in the circulation which reduces blood pressure. They may also have a 'relaxing' effect on the blood vessels which reduces the pressure within the blood vessels. Only a low dose of a diuretic is needed to treat high blood pressure. Therefore, you will not notice much diuretic effect (that is, you will not pass much extra urine). You will need a blood test before starting a diuretic to check that your kidneys are working well. You should also have a blood test to check that your blood potassium has not been affected

within 4-6 weeks of starting treatment with a diuretic. Then, a yearly blood test is usual.

BETA-BLOCKERS

Again, there are various types and brands. For example, acebutolol, atenolol, bisoprolol, metoprolol, oxprenolol, pindolol, propanaolol, sotalol, and timolol. They work by slowing the heart rate, and reducing the force of the heart. These actions lower the blood pressure. Beta-blockers are also commonly used to treat angina, and some other conditions. You should not take a beta-blocker if you have asthma, chronic obstructive pulmonary disease (COPD), or certain types of heart or blood vessel problems.

SIDE-EFFECTS

All drugs have possible side-effects, and no drug is without risk. However, most people who take drugs to lower blood pressure do not develop any side effects, or only have mild side-effects. A full list of cautions and possible side-effects is listed on the leaflet inside the drug packet.

The most common ones are:

- ACE inhibitors - sometimes cause an irritating cough.
- Angiotensin receptor blockers - sometimes cause dizziness.
- Calcium channel blockers - sometimes cause dizziness, facial flushing, swollen ankles, and constipation.
- Diuretics - can cause gout attacks in a small number of users, or can make gout worse if you already have gout. Impotence develops in some users.

- Beta-blockers - can cause cool hands and feet, poor sleep, tiredness, and impotence in some users.

If you do develop a side-effect, a different drug may suit you better. There is a lot of choice so one can usually be found to suit. See your doctor if you develop any problem which you think is due to your medication.

OTHER DRUGS FOR HIGH BLOOD PRESSURE

Apart from the five main classes of drugs listed above, sometimes other drugs are used to lower blood pressure. For example, methyldopa or alpha blockers are sometimes used if there are problems with the more commonly used drugs.

COMBINATIONS OF DRUGS

One drug can reduce high blood pressure to the target level in less than half of cases. It is common to need two or more different drugs to reduce high blood pressure to a target level. In about a third of cases, three drugs or more are needed to get blood pressure to the target level. So, for example, you may need an ACE inhibitor plus a calcium-channel blocker (and sometimes also another drug) to control your blood pressure. This is just an example, and various combinations of drugs can be used. In some cases, despite treatment, the target level is not reached. However, although to reach a target level is ideal, you will gain some benefit from any reduction of high blood pressure.

BEST DRUG OR COMBINATION OF DRUGS

The one or ones chosen may depend on such things as: if you have other medical problems; your ethnic origin; if

you take other medication; possible side-effects; your age; etc. For example: beta-blockers and calcium channel blockers can also treat angina; ACE inhibitors also treat heart failure; some drugs are not suitable if you are pregnant; some are thought to be better if you have diabetes; some tend to work better than others in people of Afro-Caribbean origin; etc. If you do not have any other medical problems that warrants a particular drug, then current UK guidelines give the following recommendations as to usual drugs that should be used. These recommendations are based on treatments and combinations of treatments that are likely to give the best control of the blood pressure with the least risk of side effects or problems.

Treatment is guided by the A/CD approach as follows:

- If you are less than 55 years old and are not of black African or Caribbean origin then initial treatment should be with 'A' (an ACE inhibitor, or an angiotensin receptor blocker if an ACE inhibitor causes problems or side effects).
- If you are 55 years or older, or are of black African or Caribbean origin then initial treatment should be with 'C' or 'D' (a calcium-channel blocker or a diuretic).
- Then, if the target blood pressure is not reached, combine 'A' with 'C' or 'D' (an ACE inhibitor or an angiotensin receptor blocker plus a calcium-channel blocker or diuretic).
- Then, if target blood pressure is still not reached, combine 'A' with 'C' and 'D' (an ACE inhibitor or an angiotensin receptor blocker, and a calcium-channel blocker, and a diuretic).

- If a fourth drug is needed to achieve the target blood pressure, consider adding of one of the following:
  - A beta-blocker
  - Another diuretic
  - An alpha-blocker

However, individuals can vary. Sometimes, if one drug does not work so well or causes side-effects, a switch to a different class of drug may work fine.

MEDICATION

In most cases, medication is needed for life. However, in *some* people whose blood pressure has been well controlled for three years or more, medication *may* be able to be stopped. In particular, in people who have made significant changes to lifestyle which can affect blood pressure (such as lost a lot of weight, or stopped heavy drinking, etc). Your doctor can advise. If you stop medication, you need regular blood pressure checks. In some cases the blood pressure remains normal. However, in others it starts to rise again. Medication can then be started again.

5

Drug Neurological Disorder

Neurological disorders are health conditions involving the nervous system. A neurological disorder is a disease or injury of the central nervous system that causes paralysis of any part of the body. Sometimes physical injury to the brain, spinal cord, or nerves can be the cause of neurological disorders. Sometimes they can result from biochemical causes. Other times, the cause may be unknown and only the effects are seen. Neurological disorders can be a sign that there is an imbalance in your system. When you have an imbalance, you are also susceptible to various diseases, which can settle in weak areas of your body.

Neurological disorders are a group of disorders that involve the central nervous system (brain, brainstem and cerebellum), the peripheral nervous system (including cranial nerves), and the autonomic nervous system (parts of which are located in both central and peripheral nervous system).

Major branches are headache, stupor and coma, dementia, seizure, sleep disorders, trauma, infections, neoplasm's, neuroophthalmology, movement disorders, demyelinating diseases, spinal cord disorders, and disorders of peripheral nerves, muscle and neuromuscular junctions. Neurological disabilities are associated with damage to the nervous system (including the brain and spinal cord) that results in the loss of some bodily or mental functions. Acquired Brain Injury (ABI), and Epilepsy are two of the most prevalent neurological disabilities. Heart attacks, infections, genetic disorders, and lack of oxygen to the brain may also result in a neurological disability.

Neurological disorders are quite diverse, chronic, challenging to treat, and often disabling. They can be caused by many different factors, including (but not limited to): inherited genetic abnormalities, problems in the immune system, injury to the brain or nervous system, or diabetes. Many mental illnesses are believed to be neurological disorders of the central nervous system, but they are classified separately. They are not traditionally listed as neurological diseases because their causes are not definitely determined as biological, although there are good reasons to suspect that bipolar disorder and schizophrenia have neuro-chemical causes. The human central nervous system consists of the brain and spinal cord. These lie in the midline of the body and are associated with the skull and vertebrae respectively. The central nervous system along with the peripheral nervous system comprises a primary division of controls that command all physical activities of a vertebrate. Neurons of the central nervous system affect consciousness

and mental activity while spinal extensions of central nervous system neuron pathways affect skeletal muscles and organs in the body.

CONDITIONS

Multiple sclerosis is a demyelinating disease, a non-contagious chronic autoimmune disorder of the central nervous system which can present with a variety of neurological symptoms occurring in attacks or slowly progressing over time. It has no cure yet and the exact cause remains unknown. Due to its effects of the nervous system, it can lead to long-term impaired mobility and disability in severe cases. Multiple sclerosis slowly progressive autoimmune disease in which the body's immune system attacks the protective myelin sheaths that surround the nerve cells of the brain and spinal cord (a process called demyelination), resulting in damaged areas that are unable to transmit nerve impulses.

Multiple sclerosis can be thought of as an inflammatory process involving different areas of the central nervous system (CNS) at various points in time. During an multiple sclerosis attack, inflammation occurs in areas of the white matter of the central nervous system (nerve fibers that are the site of multiple sclerosis lesions) in random patches called plaques. This process is followed by destruction of myelin, which insulates nerve cell fibers in the brain and spinal cord. Myelin facilitates the smooth, high-speed transmission of electrochemical messages between the brain, the spinal cord, and the rest of the body; when it is damaged, neurological transmission of messages may be slowed or blocked completely, leading to diminished or lost function.

Multiple sclerosis is a nerve disorder caused by destruction of the insulating layer surrounding neurons in the brain and spinal cord. This insulation, called myelin, helps electrical signals pass quickly and smoothly between the brain and the rest of the body. When the myelin is destroyed, nerve messages are sent more slowly and less efficiently. Patches of scar tissue, called plaques, form over the affected areas, further disrupting nerve communication. The symptoms of multiple sclerosis occur when the brain and spinal cord nerves no longer communicate properly with other parts of the body. multiple sclerosis causes a wide variety of symptoms and can affect vision, balance, strength, sensation, coordination, and bodily functions.

Multiple sclerosis affects more than a quarter of a million people in the United States. Most people have their first symptoms between the ages of 20 and 40; symptoms rarely begin before 15 or after 60. Women are almost twice as likely to get multiple sclerosis as men, especially in their early years. People of northern European heritage are more likely to be affected than people of other racial backgrounds, and multiple sclerosis rates are higher in the United States, Canada, and Northern Europe than in other parts of the world. multiple sclerosis is very rare among Asians, North and South American Indians, and Eskimos. The onset of multiple sclerosis is usually at age 20 to 40 years, and its many symptoms affect almost every system of the body. There may be visual difficulties, emotional disturbances, speech disorders, convulsions, paralysis or numbness of various regions of the body, bladder disturbances, and muscular weakness. The course of the disease varies greatly

from person to person. In some patients, the symptoms remit and return, sometimes at frequent intervals and sometimes after several years. In others the disease progresses steadily. The disease is more common in women than men, and often appears between the ages of 20 and 45. It is more frequently seen in the temperate zones, such as northern Europe, than the subtropical and tropical areas of the World. In Europe and North America multiple sclerosis is the most common cause of neurological disability in young adults, affecting 1 in 800 of the population.

Multiple sclerosis is not strictly a hereditary disease. However, multiple sclerosis is a disease influenced by a variety of factors, one of which is the genetic background of an individual. There is no single gene known to be responsible for multiple sclerosis, though a few genes have been demonstrated to increase the risk of development. Although these genes are of scientific interest and continue to play a part in research, they are not enough to diagnose an individual with multiple sclerosis.

CEREBRAL PALSY

Cerebral palsy or CP is a group of disorders associated with developmental brain injuries that occur during fetal development, birth, or shortly after birth. It is characterized by a disruption of motor skills, with symptoms such as spasticity, paralysis, or seizures. Cerebral palsy is also known as static encephalopathy and Little's disease (which is strictly speaking only the "spastic diplegia" form of CP). It is no longer considered a disease, but rather it is a chronic nonprogressive neurological disorder. The incidence is about

1.5 to 4 per 1000 live births. There is no cure, but therapy may be helpful. It has one of the highest lifetime costs of any birth defect.

The disorder is marked by several important signs. All persons with cerebral palsy developed it while the brain was under development. This limits the age at which the disorder can develop to at most 5 years old, however 80% of all cases occur before the baby reaches 1 month old. Secondly, it is a nonprogressive disorder, that is, once the damage to the brain is done no additional damage occurs. Cerebral palsy never worsens, though its symptoms may change with time. The disorder also never improves. It is a permanent disability which stays with a person their entire life. Any temporary problems would suggest a disorder other than cerebral palsy, which is why a reliable diagnosis of it can't occur until the child is four or five years old. Additionally, the disorder is characterized by disruption of the motor skills of the person. The severity in the loss of motor skills varies greatly from case to case. Lastly, even though there is a loss of motor skills, the muscles themselves are not defective. The problem lies solely in the brain's ability to control those otherwise healthy muscles.

The affected muscles of a person with CP may become rigid or excessively loose, or the person may lose control of muscles, or have problems with balance and coordination. A combination of these is also possible. The person may be primarily affected in the legs (paraplegia or diplegia), or in the arm and leg of one side of the body (hemiplegia), or all four limbs may be involved (quadriplegia). A person with CP may also be affected by a number of other problems,

including seizure disorder, visual deficits, hearing problems, mental retardation, learning disabilities, and attention-deficit/hyperactivity disorder. None of these is necessarily part of CP, however, and a person with CP may have no other impairments except for the movement disorder.

Cerebral palsy affects approximately 500,000 children and adults in the United States, and is diagnosed in more than 6,000 newborns and young children each year. Cerebral palsy is not an inherited disorder, and as of yet there is no way to predict with certainty which children will develop it. It is not a disease, and is not communicable. CP is a nonprogressive disorder, which means that symptoms neither worsen nor improve over time. However manifestation of the symptoms may become more severe over time; for example, rigidity of muscles can lead to contractures and deformities that require a variety of interventions.

HEADACHES

A headache is a condition of mild to severe pain in the head; sometimes upper back or neck pain may also be interpreted as a headache. Most headaches are due to tension, migraine, or a combination of the two. Serious underlying causes of headaches, like a tumor or a stroke, are extremely rare, despite the fact that many people worry about these possibilities.

There are three types of primary headaches: tension-type (muscular contraction headache), migraine (vascular headaches), and cluster. Virtually everyone experiences a tension-type headache at some point. An estimated 18% of American women suffer migraines, compared to 6% of men.

Cluster headaches affect fewer than 0.5% of the population, and men account for approximately 80% of all cases. Headaches caused by illness are secondary headaches and are not included in these numbers.

Headaches have a wide variety of causes, ranging from eyestrain to inflammation of the sinus cavities to life-threatening conditions such as encephalitis, brain cancer, and cerebral aneurysms. When the headache occurs in conjunction with a head injury the cause is usually quite evident; however, many causes of headaches are more elusive. The most common type of headache is a tension headache. Some people experience headaches when they are hungry or dehydrated.

In developed countries, Tension Type Headache (TTH) alone affects two-thirds of adult males and over 80% of females. Extrapolation from figures for migraine prevalence and attack incidence suggests that 3000 migraine attacks occur every day for each million of the general population. Less well recognized is the toll of chronic daily headache: up to one adult in 20 has headache every or nearly every day.

Not only is headache painful, but headache disorders are also disabling. Worldwide, according to the World Health Organization (WHO), migraine alone is 19th among all causes of years lived with disability (YLDs). Headache disorders impose recognizable burden on sufferers including sometimes substantial personal suffering, impaired quality of life and financial cost. Repeated headache attacks, and often the constant fear of the next one, damage family life, social life and employment. For example, social activity and

work capacity are reduced in almost all migraine sufferers and in 60% of TTH sufferers.

The long-term effort of coping with a chronic headache disorder may also predispose the individual to other illnesses. For example, depression is three times more common in people with migraine or severe headaches than in healthy individuals.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is an irreversible, progressive disorder in which brain cells (neurons) deteriorate, resulting in the loss of cognitive functions, primarily memory, judgment and reasoning, movement coordination, and pattern recognition. In advanced stages of the disease, all memory and mental functioning may be lost. A person with Alzheimer's disease has problems with memory, judgment, and thinking, which makes it hard for the person to work or take part in day-to-day life. The death of the nerve cells occurs gradually over a period of years.

A person with Alzheimer's disease usually has a gradual decline in mental functions, often beginning with slight memory loss, followed by losses in the ability to maintain employment, to plan and execute familiar tasks, and to reason and exercise judgment. Communication ability, mood, and personality may also be affected. Most people who have Alzheimer's disease die within eight years of their diagnosis, although that interval may be as short as one year or as long as 20 years. Alzheimer's disease is the fourth leading cause of death in adults after heart disease, cancer, and stroke.

Between two and four million Americans have Alzheimer's disease; that number is expected to grow to as many as 14 million by the middle of the 21st century as the population as a whole ages. While a small number of people in their 40s and 50s develop the disease (called early-onset Alzheimer's disease), Alzheimer's disease predominantly affects the elderly. Alzheimer's disease affects about 3% of all people between ages 65 and 74, about 19% of those between 75 and 84, and about 47% of those over 85. Slightly more women than men are affected with Alzheimer's disease, but this may be because women tend to live longer, and so there is a higher proportion of women in the most affected age groups.

People with Alzheimer's disease can develop more and more problems as time goes by. Initially, memory loss is the most noticeable, although language difficulty (for example, trouble finding words) is also seen. Poor planning, poor judgement or becoming lost even in familiar settings are all symptoms of the problem. Personality changes can develop over time, along with agitation, depression, irritability, and, rarely, aggressivity. Finally, a person with severe Alzheimer's disease will be unable to talk, unable to sit up, and unable to control bowels or bladder. Understandably, many older people - and their children - fear the development of Alzheimer's disease.

CHRONIC FATIGUE SYNDROME

Chronic fatigue syndrome (CFS) is an illness characterized by prolonged, debilitating fatigue and multiple nonspecific symptoms such as headaches, recurrent sore

throats, muscle and joint pains, memory and concentration difficulties. Profound fatigue, the hallmark of the disorder, can come on suddenly or gradually and persists or recurs throughout the period of illness. Unlike the short-term disability of say, the flu, chronic fatigue syndrome symptoms linger for at least six months and often for years. The cause of chronic fatigue syndrome remains unknown.

Chronic fatigue syndrome is a debilitating and complex disorder characterized by profound fatigue of six months or longer duration that is not improved by bed rest and that may be worsened by physical or mental activity. Persons with chronic fatigue syndrome (CFS) most often function at a substantially lower level of activity than they were capable of before the onset of illness. In addition to these key defining characteristics, patients report various nonspecific symptoms, including weakness, muscle pain, impaired memory and/or mental concentration, insomnia and post-exertional fatigue lasting more than 24 hours. In some cases, chronic fatigue syndrome can persist for years.

Chronic fatigue syndrome may occur after an infection such as a cold, bronchitis, mononucleosis, hepatitis or intestinal illness. It can start during or shortly after a period of high stress or come on gradually without any clear starting point and any obvious cause. Chronic fatigue syndrome is a flu-like condition that can drain your energy and sometimes last for years. People previously healthy and full of energy may experience extreme fatigue, weakness and headaches as well as painful joints, muscles and lymph nodes.

Chronic fatigue syndrome is the most common name for this disorder, but it also has been called chronic fatigue and

immune disorder (CFIDS), myalgic encephalomyelitis, low natural killer cell disease, post-viral syndrome, Epstein-Barr disease, and Yuppie flu. Chronic fatigue syndrome has so many names because researchers have been unable to find out exactly what causes it and because there are many similar, overlapping conditions. Reports of a CFS-like syndrome called neurasthenia date back to 1869. Later, people with similar symptoms were said to have fibromyalgia because one of the main symptoms is myalgia, or muscle pain. Because of the similarity of symptoms, fibromyalgia and chronic fatigue syndrome are considered to be overlapping syndromes.

In the early to mid-1980s, there were outbreaks of chronic fatigue syndrome in some areas of the United States. Doctors found that many people with chronic fatigue syndrome had high levels of antibodies to the Epstein-Barr virus (EBV), which causes mononucleosis, in their blood. For a while they thought they had found the culprit, but it turned out that many healthy people also had high EBV antibodies. Scientists have also found high levels of other viral antibodies in the blood of people with chronic fatigue syndrome. These findings have led many scientists to believe that a virus or combination of viruses may trigger chronic fatigue syndrome.

Chronic fatigue syndrome was sometimes referred to as Yuppie flu because it seemed to often affect young, middle-class professionals. In fact, chronic fatigue syndrome can affect people of any gender, age, race, or socioeconomic group. Although anyone can get chronic fatigue syndrome, most patients diagnosed with chronic fatigue syndrome are 25-45 years old, and about 80% of cases are in women.

Estimates of how many people are afflicted with chronic fatigue syndrome vary due to the similarity of chronic fatigue syndrome symptoms to other diseases and the difficulty in identifying it. The Centers for Disease Control and Prevention (CDC) has estimated that 4-10 people per 100,000 in the United States have chronic fatigue syndrome. According to the CFIDS Foundation, about 500,000 adults in the United States (0.3% of the population) have chronic fatigue syndrome. This probably is a low estimate since these figures do not include children and are based on the CDC definition of chronic fatigue syndrome, which is very strict for research purposes

PARKINSON'S DISEASE

Parkinson's disease is a neurodegenerative disease of the substantia nigra (an area in the basal ganglia). The disease was first discovered and its symptoms documented in 1817 (Essay on the Shaking Palsy) by the British physician Dr. James Parkinson; the associated biochemical changes in the brain of patients were identified in the 1960s. The disease is a progressive movement disorder of the extrapyramidal system, which controls and adjusts communication between neurons in the brain and muscles in the human body.

Parkinson's disease involves a breakdown of the nerve cells in the motor area of the brain. As the cells break down, there is a shortage of dopamine. Dopamine is a neurotransmitter, or chemical that carries messages to the body. When there is a shortage of dopamine, the messages that regulate movement aren't sent properly. Parkinson's disease happens when nerve cells (neurons) in a part of the

brain called the substantia nigra gradually die. These cells normally produce dopamine, a chemical that helps to relay messages between areas of the brain that control body movement. The death of cells in this area of the brain leads to abnormally low levels of dopamine, which makes it difficult for a person with Parkinson's disease to control muscle tension and muscle movement, both at rest and during periods of activity. Parkinson's disease is progressive, meaning the signs and symptoms become worse over time. But although Parkinson's may eventually be disabling, the disease often progresses gradually, and most people have many years of productive living after a diagnosis. Unlike other serious neurologic diseases, Parkinson's disease is treatable. For decades, the drug levodopa, commonly known as L-dopa, has been the mainstay of Parkinson's disease treatment. But L-dopa can cause side effects, and it may become less effective as the disease worsens, especially as new symptoms develop. In addition, responses to the drug may become more erratic over time. For that reason newer drugs are now also used, either alone or in combination with levodopa. It is estimated that 4 million people are suffering from the disease world-wide. Parkinson's disease affects all ethnic groups. Although Parkinson's disease occurs in every part of the world, because it is mainly an illness of later life, it is more common in developed countries where people live longer. The overall prevalence of Parkinson's disease in Europe is approximately 1.6 – 1.8 per 100 in persons over 65 years of age. Because of the ageing of the world population, the importance of Parkinson's disease as a public health issue is expected to increase.

CARPAL TUNNEL SYNDROME

Carpal tunnel syndrome occurs when tendons in the wrist become inflamed after being aggravated. Tendons can become aggravated when the carpals (a tunnel of bones) and the ligaments in the wrist narrow, pinching nerves that reach the fingers and the muscle at the base of the thumb. Repetitive flexing and extension of the wrist may cause a thickening of the protective sheaths that surround each of the tendons, which narrows the tunnel. Women are three times more likely to develop CTS than men, and the risk increases with age. People between the ages of 40 and 60 are more commonly affected.

Carpal tunnel syndrome is a condition that may be caused by repeatedly performing stressful motions with your hand or holding your hand in the same position for long periods of time. CTS is classified as a cumulative trauma disorder, an ailment that attacks the body's musculoskeletal system. The musculoskeletal system is made up of muscles that pull on tendons and move the bones at joints. The joints are held together by ligaments. Carpal tunnel syndrome specifically affects the sensitive nerves of, and the blood supply that feeds, the hands and wrists.

Carpus is a word derived from the Greek word "karpos" which means "wrist." The wrist is surrounded by a band of fibrous tissue which normally functions as a support for the joint. The tight space between this fibrous band and the wrist bone is called the carpal tunnel. The median nerve passes through the carpal tunnel to receive sensations from the thumb, index, and middle fingers of the hand. Many conditions can cause increased pressure within the carpal

tunnel and lead to carpal tunnel syndrome. Carpal tunnel syndrome was first described with broken wrists. A broken wrist can cause bleeding and swelling within the carpal tunnel leading to increased pressure within the carpal tunnel. Most people with carpal tunnel syndrome have no identifiable cause. It affects almost 5% of the population and is most common in middle-aged women. Carpal tunnel syndrome is diagnosed based on the complaints of the individual combined with physical tests and often electrical studies. No single test is definitive for diagnosis of carpal tunnel syndrome. Instead, the person's complaints and test findings together lead to its diagnosis.

NEUROPATHY

Neuropathy is the disease of the nervous system. Neuropathy is a disturbance in the function of a nerve or particular group of nerves. Many people who have had diabetes for a while have nerve damage. The three major forms of nerve damage are: peripheral neuropathy, autonomic neuropathy, and mononeuropathy. The most common form is peripheral neuropathy, which mainly affects the feet and legs.

Neuropathy can lead to disability, amputation, decreased ambulation as well as foot and leg ulceration because of loss or damage to nerves which feel sensation in the lower limbs. Another reason for the disability is due to the changes that can occur in the biomechanics of feet and legs, leading to an increased risk of ulcers. A diabetic person that has neuropathy increases their risks of amputation of an extremity 2 fold.

Peripheral neuropathy is a general term referring to disorders of peripheral nerves. The peripheral nervous system is made up of the nerves that branch out of the spinal cord to all parts of the body. Peripheral nerve cells have three main parts: cell body, axons, and dendrites. Any part of the nerve can be affected, but damage to axons is most common. The axon transmits signals from nerve cell to nerve cell. Most axons are surrounded by a substance called myelin, which facilitates signal transmission. Peripheral neuropathy can be associated with poor nutrition, a number of diseases, and pressure or trauma. Many people suffer from the disorder without ever identifying the cause.

Mononeuropathy involves damage or destruction of an isolated nerve or nerve group. It is a type of peripheral neuropathy (damage to nerves outside the brain and spinal cord). Mononeuropathy is most often caused by damage to a local area resulting from injury or trauma, although occasionally systemic disorders may cause isolated nerve damage (as with mononeuritis multiplex). The usual causes are direct trauma, prolonged pressure on the nerve and compression of the nerve by swelling or injury to nearby body structures. The damage includes destruction of the myelin sheath (covering) of the nerve or of part of the nerve cell (the axon). This damage slows or prevents conduction of impulses through the nerve.

Autonomic neuropathy effect nerves of body parts that we cannot consciously control. The digestive system is most often affected, especially the intestine and stomach, blood vessels and heart, urinary system. It can affect the nerves that control you tiny muscles of the eyes and sex organs. It

can also affect the return of normal blood sugars when an episode of hypoglycemia occurs. This type of nerve damage frequently results in damage that causes the inability to sense low blood sugars. To prevent autonomic neuropathy, you need to continuously keep your blood glucose levels well controlled.

In all neuropathy and neuropathic pain, there is abnormal conduction of nerve impulses from the input (usually peripheral in the extremities) to the spinal cord and brain. The pain of neuropathy is a result of the abnormal processing of nerve impulses that originate in these peripheral nerves. The terms neuropathy and peripheral neuropathy are often used interchangeably to describe the same process. Neuropathy can cause strange and extremely unpleasant sensations to arise in the affected area, including paresthesia (tingling or numbness), causalgia (burning sensations), and dysesthesia (unpleasant, burning, crawling, itchy, tingling or numb sensations)—or just plain pain.

Pain associated with neuropathy can be very intense and may be described as cutting, stabbing, crushing, burning, shooting, gnawing, or grinding. In some cases, a nonpainful stimulus (such as a feather drawn across the skin) may be perceived as excruciating, or pain may be felt even in the absence of a stimulus. If a problem with the motor nerve has continued over a length of time, muscle shrinkage (atrophy), or lack of muscle tone, may be noticeable. Autonomic nerve damage can also occur and is most noticeable when an individual stands upright and experiences difficulties such as light-headedness or changes in blood pressure. Other indicators of autonomic nerve

damage are lack of sweat, tears, and saliva; urinary retention; and impotence. In some cases, heart beat irregularities and respiratory problems can develop.

Neuropathy often results in numbness, abnormal sensations called dysthesias and allodynias that occur either spontaneously or in reaction to external stimuli, and a characteristic form of pain, called neuropathic pain or neuralgia, that is qualitatively different from the ordinary nociceptive pain one might experience from stubbing a toe or hitting a finger with a hammer. Neuropathic pain is usually perceived as a steady burning and/or "pins and needles" and/or "electric shock" sensations. The difference is due to the fact that "ordinary" pain stimulates only pain nerves, while a neuropathy often results in the firing of both pain and non-pain (touch, warm, cool) sensory nerves in the same area, producing signals that the spinal cord and brain do not normally expect to receive.

DISORDERS

A synthetic disorder is a disease caused by a different form of a gene called a variation, or an alteration of a gene called a mutation. Many diseases have a genetic aspect. Some, including many cancers, are caused by a mutation in a gene or group of genes in a person's cells. These mutations can occur randomly or because of an environmental exposure such as cigarette smoke. Other synthetic disorders are inherited. A mutated gene is passed down through a family and each generation of children can inherit the gene that causes the disease. Still other genetic disorders are due to problems with the number of packages

of genes called chromosomes. In Down syndrome, for example, there is an extra copy of chromosome 21. Both environmental and genetic factors have roles in the development of any disease. A genetic disorder is a disease caused by abnormalities in an individual's genetic material (genome).

The four different types of genetic disorders are:

1. Single-gene,
2. Multifactorial,
3. Chromosomal,
4. Mitochondrial.

1. *Single-gene (also called Mendelian or monogenic)*: This type is caused by changes or mutations that occur in the DNA sequence of one gene. Genes code for proteins, the molecules that carry out most of the work, perform most life functions, and even make up the majority of cellular structures. When a gene is mutated so that its protein product can no longer carry out its normal function, a disorder can result. There are more than 6,000 known single-gene disorders, which occur in about 1 out of every 200 births. Some examples are cystic fibrosis, sickle cell anemia, Marfan syndrome, Huntington's disease, and hereditary hemochromatosis.

   Single-gene disorders are inherited in recognizable patterns: autosomal dominant, autosomal recessive, and X-linked.

2. *Multifactorial (also called complex or polygenic)*: This type is caused by a combination of environmental

factors and mutations in multiple genes. For example, different genes that influence breast cancer susceptibility have been found on chromosomes 6, 11, 13, 14, 15, 17, and 22. Its more complicated nature makes it much more difficult to analyse than single-gene or chromosomal disorders. Some of the most common chronic disorders are multifactorial. Examples include heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes, cancer, and obesity. Multifactorial inheritance also is associated with heritable traits such as fingerprint patterns, height, eye colour, and skin colour.

3. *Chromosomal*: Chromosomes, distinct structures made up of DNA and protein, are located in the nucleus of each cell. Because chromosomes are carriers of genetic material, such abnormalities in chromosome structure as missing or extra copies or gross breaks and rejoinings (translocations) can result in disease. Some types of major chromosomal abnormalities can be detected by microscopic examination. Down syndrome or trisomy 21 is a common disorder that occurs when a person has three copies of chromosome 21.
4. *Mitochondrial*: This relatively rare type of genetic disorder is caused by mutations in the nonchromosomal DNA of mitochondria. Mitochondria are small round or rod-like organelles involved in cellular respiration and found in the cytoplasm of plant and animal cells. Each mitochondrion may contain 5 to 10 circular pieces of DNA.

6

Drug Delivery

One application of nanotechnology in medicine currently being developed involves employing nanoparticles to deliver drugs, heat, light or other substances to specific types of cells (such as cancer cells). Particles are engineered so that they are attracted to diseased cells, which allows direct treatment of those cells. This technique reduces damage to healthy cells in the body and allows for earlier detection of disease.

For example, nanoparticles that deliver chemotherapy drugs directly to cancer cells are under development. Tests are in progress for targeted delivery of chemotherapy drugs and their final approval for their use with cancer patients is pending, as explained on CytImmune Science's website. CytImmune has published the preliminary results of a Phase 1 Clinical Trial of their first targeted chemotherapy drug.

If you hate getting shots, you'll be glad to hear that oral

administration of drugs that currently are delivered by injection may be possible in many cases. The drug is encapsulated in a nanoparticle which helps it pass through the stomach to deliver the drug into the bloodstream. There are efforts underway to develop oral administration of several different drugs using a variety of nanoparticles.

THERAPY TECHNIQUES

Buckyballs may be used to trap free radicals generated during an allergic reaction and block the inflammation that results from an allergic reaction. Nanoshells may be used to concentrate the heat from infrared light to destroy cancer cells with minimal damage to surrounding healthy cells. For a good visual explanation of nanoshells, click here. Nanospectra Biosciences has developed such a treatment using nanoshells illuminated by an infrared laser that has been approved for a pilot trial with human patients.

Nanoparticles, when activated by x-rays, that generate electrons that cause the destruction of cancer cells to which they have attached themselves. This is intended to be used in place radiation therapy with much less damage to healthy tissue. Nanobiotix has released preclinical results for this technique. Aluminosilicate nanoparticles can more quickly reduce bleeding in trauma patients by absorbing water, causing blood in a wound to clot quickly. Z-Medica is producing a medical gauze that uses aluminosilicate nanoparticles.

NANOMEDICINE

Quantum Dots (qdots) may be used in the future for locating cancer tumors in patients and in the near term for

performing diagnostic tests in samples. Invitrogen's website provides information about qdots that are available for both uses, although at this time the use "in vivo" (in a living creature) is limited to experiments with lab animals. Iron oxide nanoparticles can used to improve MRI images of cancer tumors. The nanoparticle is coated with a peptide that binds to a cancer tumor, once the nanoparticles are attached to the tumor the magnetic property of the iron oxide enhances the images from the Magnetic Resonance Imagining scan.

Nanoparticles can attach to proteins or other molecules, allowing detection of disease indicators in a lab sample at a very early stage.

There are several efforts to develop nanoparticle disease detection systems underway. One system being developed by Nanosphere, Inc. uses gold nanoparticles, Nanosphere has clinical study results with their Verigene system involving it's ability to detect four different nucleic acids, while another system being developed by T2 Biosystems uses magnetic nanoparticles to identify specimens, including proteins, nucleic acids, and other materials.

MECHANISMS OF DRUGS

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

1. *Drug inactivation or modification*: *e.g.*, enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of â-lactamases.
2. *Alteration of target site*: *e.g.*, alteration of PBP — the binding target site of penicillins — in MRSA and other penicillin-resistant bacteria.

3. *Alteration of metabolic pathway*: *e.g.*, some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilising preformed folic acid.
4. *Reduced drug accumulation*: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

METABOLIC PRICE

Biological cost or *metabolic price* is a measure of the increased energy metabolism required to achieve a function. Drug resistance has a high metabolic price in pathogens for which this concept is relevant (bacteria, endoparasites, and tumor cells.) In viruses, an equivalent "cost" is genomic complexity.

TREATMENT

The chances of drug resistance can sometimes be minimised by using multiple drugs simultaneously. This works because individual mutations can be independent and may tackle only one drug at a time; if the individuals are still killed by the other drugs, then the mutations cannot persist. This was used successfully in tuberculosis. However, cross resistance where mutations confer resistance to two or more treatments can be problematic.

For antibiotic resistance, which represents a widespread problem nowadays, destroying the resistant bacteria can be achieved by phage therapy, in which specific bacteriophage (virus that kill bacteria) are being used.

MEDICINAL MYCOLOGY

For centuries, certain mushrooms have been documented as a folk medicine in China, Japan, and Russia. Although the use of mushrooms in folk medicine is largely centered on the Asian continent, people in other parts of the world like the Middle East, Poland and Belarus have been documented using mushrooms for medicinal purposes. Certain mushrooms, especially polypores like Reishi were thought to be able to benefit a wide variety of health ailments. Medicinal mushroom research in the United States is currently active, with studies taking place at City of Hope National Medical Center, as well as the Memorial Sloan–Kettering Cancer Center.

Current research focuses on mushrooms that may have hypoglycemic activity, anti-cancer activity, anti-pathogenic activity, and immune system enhancing activity. Recent research has found that the oyster mushroom naturally contains the cholesterol-lowering drug lovastatin, mushrooms produce large amounts of vitamin D when exposed to UV light, and that certain fungi may be a future source of taxol. To date, penicillin, lovastatin, ciclosporin, griseofulvin, cephalosporin, ergometrine, and statins are the most famous pharmaceuticals which have been isolated from the fungi kingdom.

MEDICINAL MUSHROOMS

Medicinal mushrooms are mushrooms used or studied as possible treatments for disease. Research indicates mushrooms have potential antiviral, antimicrobial, anticancer, antihyperglycemic, hypochole sterolemic, and

anti-inflammatory activities. The pleuromutilin antibiotics are derivatives of the mushroom isolate pleuromutilin. The mushroom isolates lentinan, PSK, and PSP, are licensed pharmaceuticals in certain countries. Fungi that do not produce mushrooms, are currently being used to manufacture paclitaxel, artemisinin, and were the original source of the first beta-lactam antibiotics, statins, immunosuppressants like ciclosporin, and antifungals like griseofulvin.

HISTORY

Mushrooms, fermentation molds, mycelia, and lichens, have a history of medicinal use spanning millennia. The mushroom with the longest record of medicinal use *Ganoderma lucidum*, is known in Chinese as líng zhî ("spirit plant"), and in Japanese as mannentake ("10,000 year mushroom"). In ancient Japan, *Grifola frondosa* was worth its weight in silver. *Inonotus obliquus* was used in Russia as early as the 16th century, and its medicinal properties were described by Nobel laureate Alexandr Solzhenitsyn. Ancient Egyptians considered mushrooms food for royalty. Ötzi the Iceman was found carrying *Fomes fomentarius* and *Piptoporus betulinus*. A Hadith states, "Truffles are manna which Allah sent to the people of Israel through Moses, and its juice is a medicine for the eyes."

DEFENSIVE ACTIVITIES

Mushrooms have antimicrobial, antiviral, and nematicidal, activities *in vitro*. Antibiotics retapamulin, tiamulin, and valnemulin, are derivatives of the mushroom isolate pleuromutilin. Plectasin is a mushroom derived

defensin antibiotic. The immunosuppressants ciclosporin, mycophenolic acid, mizoribine, the antibiotics penicillin, cephalosporins, fusafungine, usnic acid, fusidic acid, fumagillin, brefeldin A, verrucarin A, alamethicin, and the antifungals griseofulvin, echinocandins, strobilurin, azoxystrobin, caspofungin, micafungin, were discovered in fungi that do not produce mushrooms. Codinaeopsin is an antimalarial fungal isolate, and Sanofi uses fungi to manufacture artemisinin.

CHOLESTEROL BIOSYNTHESIS INHIBITORS

Pleurotus creates the statin lovastatin. A study noted an unidentified statin in *Agaricus bisporus*. Zaragozic acids were discovered in fungi, and the pravastatin precursor mevastatin, was discovered in *Penicillium*. The red yeast rice fungus *Monascus purpureus* creates lovastatin, mevastatin, and the simvastatin precursor, monacolin J. Mushroom isolate eritadenine and its 3-deaza derivatives have hypocholesterolemic activity *in vivo*.

ANTICANCER ACTIVITIES

Lentinan, PSK, and PSP, are registered anticancer immunologic adjuvants/BRMs. Irofulven and acylfulvene are anticancer derivatives of illudin S. *Clavariadelphus truncatus* creates the FTI clavaric acid. *Inonotus obliquus* creates the betulinic acid precursor betulin. *Flammulina velutipes* creates asparaginase. *Agaricus* creates conjugated linoleic acid. BMS manufactures paclitaxel using *Penicillium* and plant cell fermentation (PCF). Endophytic fungi can synthesize podophyllotoxin and camptothecin, precursors to etoposide, teniposide, topotecan, and irinotecan.

VITAMIN D AND ERGOTHIONEINE

Mushrooms exposed to UV-light create significant amounts of vitamin D_1, vitamin D_2, and vitamin D_4. This is notable, as non-animal sources of vitamin D are practically nonexistent. Although ergothioneine is only synthesized by mushrooms, other forms of fungi, and certain bacteria, human gene SLC22A4 ensures accumulation.

ANTIHYPERGLYCEMIC ACTIVITIES

Mushrooms have antihyperglycemic activity *in vivo*. *Inonotus obliquus* creates DPP-4 inhibitors. *Grifola frondosa* creates α-glucosidase inhibitors. *Trametes versicolour* creates α-amylase inhibitors, and the antihyperglycemic cyclic heptapeptide ternatin.

PSYCHOTROPIC ACTIVITIES

Over 200 species of mushrooms synthesize psilocybin, and its degradation product psilocin. Due to a chemical resemblance to serotonin, psilocin is a non-selective, partial serotonin agonist. *Claviceps* isolates and/or derivatives, were used to develop the headache medications cafergot, dihydroergotamine, methysergide, methylergometrine, the dementia medications hydergine, nicergoline, the Parkinson's disease medications lisuride, bromocriptine, cabergoline, pergolide, and the controlled substance lysergic acid diethylamide. The mycorrhizal mushroom *Polyozellus multiplex* synthesizes prolyl endopeptidase inhibitors polyozellin, thelephoric acid, and kynapcins –12, –13, –24, –28. The mycorrhizal mushroom *Boletus badius* synthesizes theanine. *Hericium erinaceus* isolates promote nerve growth factor synthesis *in vitro*.

OTHER ACTIVITIES

ACE inhibitor peptides were isolated from *Pleurotus*, *Agaricus*, *Ganoderma*, and *Flammulina*. Cordycepin, inotilone, quercinol, antcin A, and benzocamphorin F, are mushroom anti-inflammatory compounds. *Pholiota squarrosa* contains xanthine oxidase inhibitors. *Coprinopsis atramentaria* contains coprine, which metabolises to the acetaldehyde dehydrogenase inhibitor 1-aminocyclopropanol. Mushroom extracts inhibit 5-alpha reductase and/or aromatase *in vitro*. Other researched fungal isolates include, integrasone, ergosterol peroxide, enniatins, oudenone, velleral, peptaibols, kojic acid, beauvericin, pullulan, lichenin, gliotoxin, and asperlicin.

DRUGS FROM FUNGI

Fungi make an extraordinarily important contribution to managing disease in humans and other animals. At the beginning of the 21st century, Fungi were involved in the industrial processing of more than 10 of the 20 most profitable products used in human medicine. Two anti-cholesterol statins, the antibiotic penicillin and the immunosuppressant cyclosporin A are among the top 10. Each of these has a turn over in excess of $1 billion annually. Drug discovery continues. The following have recently been approved for human use: Micafungin is an antifungal agent; mycophenolate is used to prevent tissue rejection; rosuvastatin is usd to reduce cholesterol; and cefditoren as an antibiotic. Fungi are extremely useful organisms in biotechnology. Fungi construct unique complex molecules using established metabolic pathways. Different taxa produce sets of related molecules, each with slightly different

final products. Metabolites formed along the metabolic pathway may also be biologically active. In addition, the final compounds are often released into the environment. Manipulation of the genome, and environmental conditions during formation of compounds, enable the optimisation of product formation. On the negative side, single isolates of fungi in manufacture may lose their capacity to form or release the target molecules. Indeed, the target compound may only be expressed under specific conditions, or at a specific point in the life cycle of the fungus. It is amazing that so many biologically active compounds have been discovered and taken to the point where they are medically important. Indeed, attempts to 'discover' new and exciting molecules remains the core activity of many research groups. The role of fungi was established early in history. Yeasts have been used in the making of bread and alcohol since the beginning of civilization. In modern times, the discovery of penicillin marked the beginning of a new approach to microbial diseases in human health. More recent approaches include the application of hydrophobins to surfaces leading to biocompatibility of implants, and to emulsion formation improving drug delivery. The established importance of fungi is being expanding way beyond their capacity to transform and protect.

ANTIBIOTICS FROM FUNGI

In 1941, penicillin from the fungus Penicillium chrysogenum was first used successfully to treat an infection caused by a bacterium. Use of penicilin revolutionised the treatment of pathogenic disease. Many formally fatal diseases

caused by bacteria became treatable, and new forms of medical intervention were possible. When penicillin was first produced, the concentration of active ingredient was approximately 1 microgram per ml of broth solution. Today, improved strains and highly developed fermentation technologies produce more than 700 micrograms per ml of active ingredient. In the early broths, several closely related molecules were present. These molecules are beta lactam rings fused to five-membered thiazolidine rings, with a side chain. The side chain can be chemically modified to provide slightly different properties to the compound. The natural penicillins have a number of disadvantages. They are destroyed in the acid stomach, and so cannot be used orally. They are sensitive to beta lactamases, which are produced by resistant bacteria, thus reducing their effectiveness. Also, they only act on gram positive bacteria.

Modifications to manufacturing conditions have resulted in the development of oral forms. However, antibiotic resistance among bacteria is becoming an extremely important aspect determining the long-term use of all antibiotics. Cephalosporins also contain the beta lactam ring. The original fungus found to produce the compounds was a Cephalosporium, hence the name. As with penicillin, the cephalosporin antibiotics have a number of disadvantages. Industrial modification of the active ingredients has reduced these problems.

The only broadly useful antifungal agent from fungi is griseofulvin. The original source was Penicillium griseofulvin. Griseofulvin is fungistatic, rather than fungicidal. It is used for the treatment of dermatophytes, as it accumulates in

the hair and skin following topical application. More recently, several new groups have been developed. Strobilurins target the ubihydroquinone oxidation centre, and in mammals, the compound from fungi is immediately excreted. Basidiomycetes, especially from tropical regions, produce an enormous diversity of these compounds. Sordarins are structurally complex molecules that show a remarkably narrow range of action against yeasts and yeast-like fungi. The compounds inhibit protein biosynthesis and so may become important agents against a number of fungal pathogens of humans. Echinocandins are cyclic peptides with a long fatty acid side chain. They target cell wall formation. Semi-synthetic members of the group of compounds include pneumocandins which are in use in humans.

IMMUNE SUPPRESSANTS

Cyclosporin A is a primary metabolite of several fungi, including Trichoderma polysporum and Cylindrocarpon lucidum. Cyclosporin A has proven to be a powerful immunosuppressant in mammals, being widely used during and after bone marrow and organ transplants in humans.

Cyclosporin A is a cyclic peptide consisting of 11 mainly hydrophobic amino acids. Its inhibition of lymphocytes was first discovered during the 1970s. Subsequently, the mode of action was elucidated. Cyclosporin A binds to a cytosolic protein called cyclophilin. Cyclophilin is found amongst many different organisms and its form appears highly conserved. Cyclophilin is involved with folding the protein ribonuclease. However, the Cyclosporin A/cyclophilin complex also binds

to calcineurin. Calcineurin dephosphorylates a transcription factor, thereby triggering transcription of numerous genes associated with T cell proliferation. When the complex binds to calcineurin, T cell proliferation is suppressed. The inhibition of T cells proliferation results in the suppression of the activation process associated with invasion by foreign bodies. As a consequence, transplant tissues, which are foreign bodies, are not rejected. Calcineurin is also highly conserved amongst phylogenetically diverse organisms. In fungi such as the human pathogen Cryptococcus neoformans, calcineurin is necessary for recovery from cell cycle arrest, growth in hypertonic solutions and regulation of the calcium pump. Thus the interaction of the Cyclosporin A/cyclophilin complex with calcineurin in Cryptococcus will result in death of the pathogen. However, in humans, cyclosporin also suppresses the immune system. The side effect is an unacceptable risk, and Cyclosporin A is not used as a fungicide in humans at present. Gliotoxins also have immunological and antibiotic activity. Produced by many fungi including Aspergillus fumigatus, gliotoxins belong to a class of compounds called epipolythiodioxopiperazines. The antibiotic activity is widely recognised and considered uninteresting. However, its effect on the immune system, especially macrophages, is being re-examined. A wide range of other compounds with antibiotic activity are also known. They have been rejected for use in medicine because of unwanted side effects, or instability of the active compound.

ERGOT ALKALOIDS

Claviceps purpurea is the causal agent of St Anthonies fire, a scourge of the middle ages when ergots contaminated flour.

The ergots contain many alkaloids. Their effects are quite variable. They act on the sympathetic nervous system resulting in the inhibition of noradrenaline and sclerotin, causing dilation of blood vessels. They also act directly on the smooth muscles of the uterus causing contractions, thus their early use to induce abortion. Their strongest effect is intoxication, caused by lysergic acid amides, one of which is the recreational (and illegal) drug, LSD. Ergot alkaloids have a number of medicinal uses. Perhaps the most widespread use is in the treatment of migraines. The vasodilator activity reduces tension during an attack. The drugs also reduce blood pressure, though with untoward side effects. Alkaloids are now produced in culture by strains of C. fusiformis and C. paspalii.

STATINS

Aspergillus terreus, a soil-borne fungus, produces a secondary metabolite called lovastatin and Phoma sp produces squalestatin. Statins have been used to reduce or remove low density lipoproteins from blood vessels in humans. In fact, the compounds all act via an enzyme in the liver that makes cholesterol, lovastatin inhibits HMG CoA reductase and squalestatin inhibits squalene synthase. By blocking the enzyme, the body removes cholesterol complexes from the inside of blood vessels. This has the effect of reducing or removing blockages in arteries, and thereby reducing the chance of a heart attack, strokes and diabetes. In addition, statins have been implicated in attracting stem cells to damaged tissues.

The stem cells then appear to regenerate the tissue. Some statins induce problems. One form of the drug has been

associated with muscle wastage. Others appear to lack side effects and have been recommended for wide spread use to control heart disease.

MEDICINAL FUNGI

AGARICUS SUBRUFESCENS

Agaricus subrufescens (syn. Agaricus blazei, Agaricus brasiliensis or Agaricus rufotegulis) is a species of mushroom, commonly known as almond mushroom, mushroom of the sun, God's mushroom, mushroom of life, royal sun agaricus, jisongrong or himematsutake and by a number of other names. Agaricus subrufescens is a choice edible, with a somewhat sweet taste and fragrance of almonds. The fungus is also well known as a medicinal mushroom, for its purported medicinal properties, due to research which indicates it may stimulate the immune system.

TAXONOMY

Agaricus subrufescens was first described by the American botanist Charles Horton Peck in 1893. During the late 19th and early 20th centuries, it was cultivated for the table in the eastern United States. It was discovered again in Brazil during the 1970s, and misidentified as *Agaricus blazei* Murrill, a species originally described from Florida. It was soon marketed for its purported medicinal properties under various names, including ABM (for *Agaricus blazei* Murill), *cogumelo do sol* (mushroom of the sun), *cogumelo de Deus* (mushroom of God), *cogumelo de vida* (mushroom

of life), *himematsutake*, royal sun agaricus, *Mandelpilz*, and almond mushroom. In 2002, Didukh and Wasser correctly rejected the name *A. blazei* for this species, but unfortunately called the Brazilian fungus *A. brasiliensis*, a name that had already been used for a different species, *Agaricus brasiliensis* Fr. (1830). Richard Kerrigan undertook genetic and interfertility testing on several fungal strains, and showed that samples of the Brazilian strains called *A. blazei* and *A. brasiliensis* were genetically similar to, and interfertile with, North American populations of *Agaricus subrufescens*. These tests also found European samples called *A. rufotegulis* to be of the same species. Because *A. subrufescens* is the oldest name, it has taxonomical priority. Note that *Agaricus blazei* Murrill is a perfectly valid name, but for a completely different mushroom. *Agaricus silvaticus* Schaeff. is also a perfectly valid name for a common, north temperate, woodland mushroom. Neither is a synonym of *Agaricus subrufescens*.

DESCRIPTION

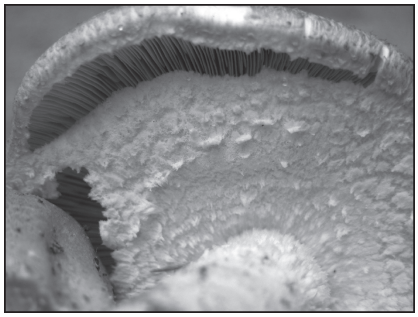


Fig. The Floccose Stipe and Annulus of *A. Subrufescens*

The cap is initially hemispherical, later becoming convex, with a diameter of 5 to 18 cm (2.0 to 7.1 in). The cap surface is covered with silk-like fibres, although in maturity it develops small scales (squamulose). The colour of the cap may range

from white to grayish or dull reddish-brown; the cap margin typically splits with age. The flesh of *A. subrufescens* is white, and has the taste of "green nuts", with the odour of almonds. The gills are not attached to the stalk (free), narrow, and crowded closely together. They start out whitish in colour, then later pinkish and finally black-brown as the spores mature. Spores are ellipsoid, smooth, dark purplish-brown when viewed microscopically, with dimensions of 6–7.5 by 4–5 μm. The stipe is 6 to 15 cm (2.4 to 5.9 in) by 1 to 1.5 cm (0.4 to 0.6 in) thick, and bulbous at the base. Initially solid, the stipe becomes hollow with age; it is cottony (floccose) to scaly towards the base. The annulus is abundant and double-layered; it is bent downwards towards the stem, smooth and whitish on the upper side, and covered with cottony scales on the lower side.

DISTRIBUTION AND HABITAT

Agaricus subrufescens forms fruitbodies singly or in clusters in leaf litter in rich soil, often in domestic habitats. Originally described from the northeastern United States and Canada, it has been found growing in California, Hawaii, Great Britain, the Netherlands, Taiwan, Philippines and Brazil.

EDIBILITY

AROMA

Agaricus subrufescens is a choice edible, with a somewhat sweet taste and fragrance of almonds. The almond smell of the mushroom is mostly due to the presence of benzaldehyde, benzyl alcohol, benzonitrile, and methyl benzoate.

COMMERCIAL USE

Because *Agaricus subrufescens* contains a high level of beta glucans, compounds known for stimulating the immune system, the fungus is used in oncological therapy in Japan and Brazil. In addition to beta glucans, the mushroom's effect on the immune system is believed to be due to other polysaccharides, such as alpha glucans. In Japan, *Agaricus subrufescens* is sold under the brand names KingAgaricus 100, Sen-Sei-Ro Gold, and ABMK, and is used by an estimated 500,000 people In Japan, *Agaricus subrufescens* is also the most popular complementary and alternative medicine used by cancer patients.

Although *Agaricus subrufescens* is cultivated in the United States, the largest exporters are China and Brazil. As noted in a scientific review of *A. subrufescens* research, the range of quality in *A. subrufescens* cultivation can affect the mushroom's ability to impact cells of the immune system. Recently, Watanabe *et al.* published a report in the *Biological and Pharmaceutical Bulletin* on a novel hybrid of *A. subrufescens* called *Basidiomycetes-X* (BDM-X) and a US patent was issued on a novel hybrid of the *A. subrufescens* edible mushroom which was crossbred (hybridised) with another medicinal mushroom, resulting in a new hybrid claimed to possess 10 to 3000 times the potency of similar but unpatented mushrooms.

RESEARCH

Many researchers have studied *Agaricus subrufescens*, as well as other medicinal mushrooms for close to 50 years, due to laboratory tests which show they may stimulate immune system cells and the production of immune system

cytokines. Below is a summary of this research, which is often based on animal or cellular models.

IMMUNE SYSTEM

Cellular and animal research has shown *A. subrufescens* may stimulate immune system cells and the production of cytokines, such as interferons and interleukins (reviewed by G. Hetland).

DIRECT ANTIVIRAL PROPERTIES

Agaricus subrufescens mushrooms are known to have antiviral properties in cell culture. The ability of *Agaricus subrufescens* to inhibit viruses in the human body has not been studied.

OTHER POSSIBLE EFFECTS

Besides evidence *Agaricus subrufescens* may up-regulate the immune system, additional research suggests the mushroom has a beneficial effect on cholesterol, inhibiting pathogenic factors, and inhibiting angiogenesis. Limited clinical and animal research suggests *Agaricus subrufescens* consumption may lower blood glucose levels and improve insulin resistance. Dietary studies have listed *A. subrufescens* as a non-animal source of conjugated linoleic acid (CLA), which contains combined trans- and a far greater amount of cis-fat isomers, the latter which is believed to be responsible for an increase in the metabolising of fatty tissue in the body (resting metabolic rate) and an increase in the development of muscle mass (which burns more calories than fat mass), resulting in healthy weight loss and better overall health.

ANTRODIA

Antrodia is a genus of fungi in the family Fomitopsidaceae. Antrodia species have fruiting bodies that typically lie flat or spread out on the growing surface, with the hymenium exposed to the outside; the edges may be turned so as to form narrow brackets. Most species are found in temperate and boreal forests, and cause brown rot.

DESCRIPTION

Antrodia are effused-resupinate, that is, they lie stretched out on the growing surface with the hymenium exposed on the outer side, but turned out at the edges to form brackets. When present, these brackets are typically white or pale brown. The pores on the surface of the hymenium may be round or angular. The context is white or pale. All species cause brown-rot. Typically, basidiospores are thin-walled, cylindrical, and narrowly ellipsoidal or fusiform in shape. Most species grow on the wood of coniferous trees, except for *A. albida*, with grows the dead wood on deciduous trees.

PHYLOGENY

In order to reliably identify the various species and strains of medicinal *Antrodia*, genetic markers have been developed and phylogenetic analyses performed. This analysis showed that there are three distinct phylogenetic lineages with the *Antrodia* genus.

CLASSIFICATION

The modern definition of the genus was described by Gilbertson and Ryvarden (1986) in their book *North American Polypores*.

DISTRIBUTION

Roughly twenty-nine species are known from Europe, 21 species in North America, and 18 species in East Asia, although more new species have been reported since the time of these publications.

SPECIES

There are approximately 50 *species in this genus*: Antrodia albida, Antrodia albidoides, Antrodia albobrunnea, Antrodia alpina, Antrodia aurantia, Antrodia bondartsevae, Antrodia calceus, Antrodia carbonica, Antrodia cinnamomea, Antrodia citrina, Antrodia conchata, Antrodia crassa, Antrodia daedaleiformis, Antrodia destructor, Antrodia eutelea, Antrodia ferox, Antrodia flava, Antrodia formosana, Antrodia gossypina, Antrodia gossypium, Antrodia heteromorpha, Antrodia infirma, Antrodia juniperina, Antrodia lalashana, Antrodia leucaena, Antrodia lindbladii, Antrodia luteola, Antrodia macra, Antrodia macrospora, Antrodia malicola, Antrodia mellita, Antrodia oleracea, Antrodia pictilis, Antrodia pini-cubensis, Antrodia plicata, Antrodia porothelioides, Antrodia primaeva, Antrodia pseudosinuosa, Antrodia pulvinascens, Antrodia ramentacea, Antrodia rupamii, Antrodia sandaliae, Antrodia saxonica, Antrodia serialiformis, Antrodia serialis, Antrodia serpens, Antrodia sinuosa, Antrodia sitchensis, Antrodia sordida, Antrodia stratosa, Antrodia subalbidoides, Antrodia submalicola, Antrodia subramentacea, Antrodia taxa, Antrodia terrei, Antrodia variiformis, Antrodia xantha.

ASTRAEUS HYGROMETRICUS

Astraeus hygrometricus, commonly known as the hygroscopic earthstar, the barometer earthstar, or the false

earthstar, is a species of fungus in the Diplocystaceae family. Young specimens resemble a puffball when young and unopened. In maturity, the mushroom displays the characteristic earthstar shape that is a result of the outer layer of fruit body tissue splitting open in a star-like manner. The false earthstar is an ectomycorrhizal species that grows in association with various trees, especially in sandy soils. *A. hygrometricus* has a cosmopolitan distribution, and is common in temperate and tropical regions. Its common names refer to the fact that it is hygroscopic (water-absorbing), and can open up its rays to expose the spore sac in response to increased humidity, and close them up again in drier conditions. The rays have an irregularly cracked surface, while the spore case is pale brown and smooth with an irregular slit or tear at the top. The gleba is white initially, but turns brown and powdery when the spores mature. The spores are reddish-brown, roughly spherical with minute warts, measuring 7.5–11 micrometers in diameter.

Despite a similar overall appearance, *A. hygrometricus* is not related to the true earthstars of genus *Geastrum*, although historically, they have been taxonomically confused. The species was first described by Christiaan Hendrik Persoon in 1801 as *Geastrum hygrometricus*. In 1885, Andrew P. Morgan proposed that differences in microscopic characteristics warranted the creation of a new genus *Astraeus* distinct from *Geastrum*; this opinion was not universally accepted by later authorities. Several Asian populations formerly thought to be *A. hygrometricus* were renamed in the 2000s once phylogenetic analyses revealed

they were unique *Astraeus* species, including *A. asiaticus* and *A. odoratus*. Research has revealed the presence of several bioactive chemical compounds in the fruit bodies. North American field guides typically rate *A. hygrometricus* as inedible.

DRUGS AND DEVELOPMENT

The traditional approach to development has been to stimulate economic growth through investment in infrastructure, support for institution building and promotion of economic reform. These measures to generate economic growth are designed to increase incomes generally, with the population and government having more material resources with which to meet their needs.

In addition, development programmes have also frequently incorporated a basic needs strategy that emphasizes the putting into place of governmental programmes to ensure elementary hygiene, clean drinking water, primary health care, education and literacy. Economic growth and basic needs strategies have been pursued simultaneously by a number of development assistance organizations at the national and international level. Historically, drug control and drug abuse considerations were not often viewed as issues of primary concern for development planners, even in the context of programmes following a basic needs strategy. This is because many development assistance programmes were devoted either to promoting economic growth or to some aspect of basic needs of the population related to, inter alia, health or education. To some extent, there has been limited recognition that drug-related issues should be taken into

account, particularly when an entire country or a specific region of a country has an influential illicit drug industry. The importance of production of illicit drugs to an economy will vary significantly from country to country. As noted earlier, income from the drug trade is exorbitant in some nations.

Speaking of Colombia, a country expert estimates that businessmen of the illegal drugs industry would have "huge combined drug and capital income relative to the size of the country's economy". He further states that "between 1976 and 1986 gross private fixed investment ranged from $1.6 to $3.7 billion and averaged $2.8 billion, figures that clearly indicate that the illegal businessmen had the capacity to invest in Colombia an amount as large as what official data attributes to the whole private sector of the country". This view of the impact of the drug trade is not atypical.

The United States Office of Technology Assessment has found that in Bolivia, the coca economy annually generates as much foreign exchange as all other exports combined. It has also found that a significant number of persons are employed in the coca economy: 7 per cent of the labour force in 1990, although this figure is significantly less than the 20 per cent estimated to have been employed in coca in the late 1980s. The economic costs of drug abuse can be categorized as direct and indirect. Direct costs involve increased costs of police, courts, military, treatment programmes, welfare payments to drug addicts and their families, as well as increased security measures by businesses. Indirect economic costs include the displacement of legal industries; diminished control over the economy; spending money for

drugs and inappropriate use of money gained from drug sales; and fiscal problems related to the inability to tax the drug economy. Concerning displacement of legal industries due to the cocaine trade, commentators have found that the cocaine industry in a developing country" can create such a high inflow of foreign exchange that dollars become cheap relative to the local currency.

This reduces the competitiveness of local products in both foreign and domestic markets". Diminished control over an economy occurs in several ways. Because money generated by illicit activities does not normally enter into the formal economic process of a country, macroeconomic planning is not possible for these funds. Hence, a source of serious error is introduced into estimates of national income and expenditure. In speaking of the need for improved information on the illegal sector, a country expert has concluded that "large financial flows are not accounted for and the enormous sums generated by the opiate industry fail to show up in investment rates. This allows a general conclusion that most investment has been unproductive and used for speculative purposes. However, an increasing part of the GNP is produced outside official accounts and thus outside intervention possibilities. This inevitably contributes to skewed macro-planning". Moreover, control over expenditures is reduced when governments have to devote resources to protect the financial and banking systems against subversion through additional banking regulation, increased reporting and other anti-money-laundering measures.

It has also been found that the poor often spend a greater percentage of their income on drugs than middle- or upper-

income persons, and at a much greater cost to their families. A country expert 9 on Pakistan has found that the "bulk of consumer expenditures on heroin are made by lower income groups, while the income generated by the heroin industry mainly flows to upper and middle income groups ". The poorest 20 per cent of the population represent nearly one-half of the drug abusers, primarily heroin addicts, according to the National Survey of Drug Abuse. The lowest income groups are most frequently represented; the highest income groups are somewhat below them; and the middle income group have the least number of drug users in Pakistan. Drug traffickers, however, do not normally put their illicit profits into productive enterprises. Nor is employment in the illicit drugs industry a significant means of putting money back into the community. According to this expert, the drug industry provides employment for less than 1 per cent of the labour force of Pakistan.

A very important indirect cost of the drug industry is a result of the fact that governments are not able to tax it. In such a case, governments have no choice but to increase taxes on those who can be expected to pay.

However, the externalities of the drug industry, *i.e.* the hidden economic and social costs of illicit drug production and trafficking, cannot be charged back to those involved. They are, therefore, an added burden to the law-abiding population. Experts consider cocaine a good example of market failure, a situation where markets encourage behaviour that is unprofitable for society and discourage behaviour that is better for development: "Market failure occurs when there is a difference between the costs of an

action for an individual and the cost of that action for society.

In the case of cocaine, market failure occurs -the market encourages cocaine production even though it may be bad for society -because the roughly 5% to 15% of the national population involved in the cocaine industry do not bear many of the costs they impose on society". This situation of market failure applies not only to cocaine production and trafficking but also to the production and sales of other illicit drugs.

ANTI-MICROBIAL

A welcome idea in the early study stages is the elimination of bacterial infections in a patient within minutes, instead of delivering treatment with antibiotics over a period of weeks. You can read about design analysis for the antimicrobial nanorobot used in such treatments in the following article: Microbivores: Artifical Mechanical Phagocytes using Digest and Discharge Protocol.

CELL REPAIR

Nanorobots could actually be programmemed to repair specific diseased cells, functioning in a similar way to antibodies in our natural healing processes.

Read about design analysis for one such cell repair nanorobot in this article: The Ideal Gene Delivery Vector: Chromallocytes, Cell Repair Nanorobots for Chromosome Repair Therapy

MEDICINE: RESOURCES

National Cancer Institute Alliance for Nanotechnology in Cancer; This alliance includes a Nanotechnology

Characterization Lab as well as eight Centres of Cancer Nanotechnology Excellence.

ELECTRONICS

Nanoelectronics holds some answers for how we might increase the capabilities of electronics devices while we reduce their weight and power consumption. Some of the nanoelectronics areas under development, which you can explore in more detail by following the links provided in the next section, include the following topics. Improving display screens on electronics devices. This involves reducing power consumption while decreasing the weight and thickness of the screens.

Increasing the density of memory chips. Researchers are developing a type of memory chip with a projected density of one terabyte of memory per square inch or greater. Reducing the size of transistors used in integrated circuits. One researcher believes it may be possible to "put the power of all of today's present computers in the palm of your hand".

APPLICATIONS UNDER DEVELOPMENT

Using MEMS techniques to control an array of probes whose tips have a radius of a few nanometers. These probes are used to write and read data onto a polymer film, with the aim of producing memory chips with a density of one terabyte per square inch or greater. Combining gold nanoparticles with organic molecules to create a transistor known as a NOMFET (Nanoparticle Organic Memory Field-Effect Transistor). Using carbon nanotubes to direct electrons to illuminate pixels, resulting in a lightweight, millimeter thick "nanoemmissive" display panel.

Making integrated circuits with features that can be measured in nanometers (nm), such as the process that allows the production of integrated circuits with 45 nm wide transistor gates. Using nanosized magnetic rings to make Magnetoresistive Random Access Memory (MRAM) which research has indicated may allow memory density of 400 GB per square inch. Developing molecular-sized transistors which may allow us to shrink the width of transistor gates to approximately one nm which will significantly increase transistor density in integrated circuits.

SPACE

Nanotechnology may hold the key to making space-flight more practical. Advancements in nanomaterials make lightweight solar sails and a cable for the space elevator possible. By significantly reducing the amount of rocket fuel required, these advances could lower the cost of reaching orbit and traveling in space.

NANOTECHNOLOGY AND SPACEFLIGHT

Nanotechnology may hold the key to making spaceflight more practical. Advancements in nanomaterials make lightweight solar sails and a cable for the space elevator possible. By significantly reducing the amount of rocket fuel required, these advances could lower the cost of reaching orbit and traveling in space. In addition, new materials combined with nanosensors and nanorobots could improve the performance of spaceships, spacesuits, and the equipment used to explore planets and moons, making nanotechnology an important part of the 'final frontier.'

SPACEFLIGHT

Including layers of bio-nano robots in spacesuits. The outer layer of bio-nano robots would respond to damages to the spacesuit, for example to seal up punctures. An inner layer of bio-nano robots could respond if the astronaut was in trouble, for example by providing drugs in a medical emergency. Deploying a network of nanosensors to search large areas of planets such as Mars for traces of water or other chemicals. To read more about this, see page 27 of this report on Bio-Nano-Machines for Space Applications. Employing materials made from carbon nanotubes to reduce the weight of spaceships while retaining or even increasing the structural strength.

Producing thrusters for spacecraft that use MEMS devices to accelerate nanoparticles. This should reduce the weight and complexity of thruster systems used for interplanetary missions.

One cost-saving feature of these type of thrusters is their ability to draw on more or less of the MEMS devices depending upon the size and thrust requirement of the spacecraft, rather than designing and building different engines for different size spacecraft. Using carbon nanotubes to build lightweight solar sails that use the pressure of light from the sun reflecting on the mirror-like solar cell to propel a spacecraft. This solves the problem of having to lift enough fuel into orbit to power spacecraft during interplanetary missions. Working with nanosensors to monitor the levels of trace chemicals in spacecraft to monitor the performance of life support systems.

RESEARCH

The Centre for Nanotechnology at NASA Ames is looking at how nanotechnology can be used to reduce the mass, volume, and power consumption of a wide range of spacecraft systems including sensors, communications, navigation, and propulsion systems. The Johnson Space Centre Nano Materials Project is working on nanotube composites with the aim of reducing spacecraft weight. The LiftPort Group is dedicated to making the space elevator reality. Their target date is October, 2031. The Space Nanotechnology Labouratory at MIT is developing high performance instrumentation for use on spaceflights.

FOOD

Nanotechnology is having an impact on several aspects of food science, from how food is grown to how it is packaged. Companies are developing nanomaterials that will make a difference not only in the taste of food, but also in food safety, and the health benefits that food delivers. Nanotechnology is having an impact on several aspects of food science, from how food is grown to how it is packaged. Companies are developing nanomaterials that will make a difference not only in the taste of food, but also in food safety, and the health benefits that food delivers.

Clay nanocomposites are being used to provide an impermeable barrier to gasses such as oxygen or carbon dioxide in lightweight bottles, cartons and packaging films. Storage bins are being produced with silver nanoparticles embedded in the plastic. The silver nanoparticles kill bacteria from any material that was previously stored in the bins,

minimizing health risks from harmful bacteria. Nanoparticles are being developed that will deliver vitamins or other nutrients in food and beverages without affecting the taste or appearance. These nanoparticles actually encapsulate the nutrients and carry them through the stomach into the bloodstream. Researchers are using silicate nanoparticles to provide a barrier to gasses (for example oxygen), or moisture in a plastic film used for packaging. This could reduce the possibly of food spoiling or drying out.

Zinc oxide nanoparticles can be incorporated into plastic packaging to block UV rays and provide anti bacterial protection, while improving the strength and stability of the plastic film. Nanosensors are being developed that can detect bacteria and other contaminates, such as salmonella, at a packaging plant. This will allow for frequent testing at a much lower cost than sending samples to a lab for analysis. This point-of-packaging testing, if conducted properly, has the potential to dramatically reduce the chance of contaminated food reaching grocery store shelves. Research is also being conducted to develop nanocapsules containing nutrients that would be released when nanosensors detect a vitamin deficiency in your body. Basically this research could result in a super vitamin storage system in your body that delivers the nutrients you need, when you need them.

"Interactive" foods are being developed by Kraft that would allow you to choose the desired flavour and Colour. Nanocapsules that contain flavour or Colour enhancers are embedded in the food; inert until a hungry consumer triggers them. The method hasn't been published, so it will be interesting to see how this particular trick is accomplished.

Researchers are also working on pesticides encapsulated in nanoparticles; that only release pesticide within an insect's stomach, minimizing the contamination of plants themselves. Another development being persued is a network of nanosensors and dispensers used throughout a farm field. The sensors recognize when a plant needs nutrients or water, before there is any sign that the plant is deficient. The dispensers then release fertilizer, nutrients, or water as needed, optimizing the growth of each plant in the field one by one.

FUEL CELLS

Nanotechnology is being used to reduce the cost of catalysts used in fuel cells to produce hydrogen ions from fuel such as methanol and to improve the efficiency of membranes used in fuel cells to separate hydrogen ions from other gases such as oxygen.

Catalysts are used with fuels such as hydrogen or methanol to produce hydrogen ions. Platinum, which is very expensive, is the catalyst typically used in this process. Companies are using nanoparticles of platinum to reduce the amount of platinum needed, or using nanoparticles of other materials to replace platinum entirely and thereby lower costs.

Fuel cells contain membranes that allow hydrogen ions to pass through the cell but do not allow other atoms or ions, such as oxygen, to pass through. Companies are using nanotechnology to create more efficient membranes; this will allow them to build lighter weight and longer lasting fuel cells. Small fuel cells are being developed that can be used to replace batteries in handheld devices such as PDAs or

laptop computers. Most companies working on this type of fuel cell are using methanol as a fuel and are calling them DMFC's, which stands for direct methanol fuel cell. DMFC's are designed to last longer than conventional batteries. In addition, rather than plugging your device into an electrical outlet and waiting for the battery to recharge, with a DMFC you simply insert a new cartridge of methanol into the device and you're ready to go.

Fuel cells that can replace batteries in electric cars are also under development. Hydrogen is the fuel most researchers propose for use in fuel cell powered cars. In addition to the improvements to catalysts and membranes discussed above, it is necessary to develop a lightweight and safe hydrogen fuel tank to hold the fuel and build a network of refueling stations. To build these tanks, researchers are trying to develop lightweight nanomaterials that will absorb the hydrogen and only release it when needed.

SOLAR CELLS

Companies have developed nanotech solar cells that can be manufactured at significantly lower cost than conventional solar cells.

SOLAR CELLS AND NANOTECHNOLOGY

Reduced manufacturing costs as a result of using a low temperature process similar to printing instead of the high temperature vacuum deposition process typically used to produce conventional cells made with crystalline semiconductor material.

Reduced installation costs achieved by producing flexible rolls instead of rigid crystalline panels. Cells made from

semiconductor thin films will also have this characteristic. Currently available nanotechnology solar cells are not as efficient as traditional ones, however their lower cost offsets this. In the long term nanotechnology versions should both be lower cost and, using quantum dots, should be able to reach higher efficiency levels than conventional ones.

Titanium dioxide nanotubes filled with a polymer to form low cost solar cells Combining carbon nanotubes, bucky-balls and polymers to produce inexpensive solar cells that can be formed by simply painting a surface. Nanoparticles in plastic film to form solar cells that can be incorporated into cases for devices such as mobile phones and laptop computers. Semiconductor nanoparticles applied in a low temperature printing process that results in low cost solar cells.

BATTERIES

Companies are currently developing batteries using nanomaterials. One such battery will be a good as new after sitting on the shelf for decades. Another battery can be recharged significantly faster than conventional batteries.

NANOTECHNOLOGY BATTERY (NANO BATTERY)

HOW CAN NANOTECHNOLOGY IMPROVE BATTERIES

Using nanotechnology in the manufacture of batteries offers the following benefits: Increasing the available power from a battery and decreasing the time required to recharge a battery. These benefits are achieved by coating the surface of an electrode with nanoparticles. This increases the surface

area of the electrode thereby allowing more current to flow between the electrode and the chemicals inside the battery. This technique could increase the efficiency of hybrid vehicles by significantly reducing the weight of the batteries needed to provide adequate power. Increasing the shelf life of a battery by using nanomaterials to separate liquids in the battery from the solid electrodes when there is no draw on the battery. This separation prevents the low level discharge that occurs in a conventional battery, which increases the shelf life of the battery dramatically.

Long shelf life battery uses "nanograss" to separate liquid electrolytes from the solid electrode until power is needed. Lithium ion batteries with nanoparticle (Nanophosphate™) electrodes that meet the safety requirements for electric cars while improving the performance. Lithium ion batteries with electrodes made from nano-structured lithium titanate that significantly improves the charge/discharge capability at sub freezing temperatures as well as increasing the upper temperature limit at which the battery remains safe from thermal runaway. Ultracapacitors using nanotubes may do even better than batteries in hybrid cars. Ultracapacitor using single atom thick graphene sheets to store electrical charge.

FUELS

Nanotechnology can address the shortage of fossil fuels such as diesel and gasoline by making the production of fuels from low grade raw materials economical, increasing the mileage of engines, and making the production of fuels from normal raw materials more efficient. Check our Nanotech in Fuels page for details.

FUEL AND NANOTECHNOLOGY

How can nanotechnology improve fuel availability? Nanotechnology can address the shortage of fossil fuels such as diesel and gasoline by: Making the production of fuels from low grade raw materials economical Increasing the mileage of engines Making the production of fuels from normal raw materials more efficient Nanotechnology can do all this by increasing the effectiveness of catalysts. Catalysts can reduce the temperature required to convert raw materials into fuel or increase the Percentage of fuel burned at a given temperature. Catalysts made from nanoparticles have a greater surface area to interact with the reacting chemicals than catalysts made from larger particles. The larger surface area allows more chemicals to interact with the catalyst simultaneously, which makes the catalyst more effective. This increased effectiveness can make a process such as the production of diesel fuel from coal more economical, and enable the production of fuel from currently unusable raw materials such as low grade crude oil. Nanotechnology, in the form of genetic engineering, can also improve the performance of enzymes used in the conversion of cellulose into ethanol. Currently ethanol added to gasoline in the United States is made from corn, which is driving up the price of corn. The plan is to use engineered enzymes to break down cellulose into sugar, is fermented to turn the sugar into ethanol. This will allow material that often goes to waste, such as wood chips and grass to be turned into ethanol.

BETTER AIR QUALITY

Nanotechnology can improve the performance of catalysts used to transform vapours escaping from cars or industrial plants into harmless gasses. That's because catalysts made

from nanoparticles have a greater surface area to interact with the reacting chemicals than catalysts made from larger particles. The larger surface area allows more chemicals to interact with the catalyst simultaneously, which makes the catalyst more effective.

HOW CAN NANOTECHNOLOGY REDUCE AIR POLLUTION

There are two major ways in which nanotechnology is being used to reduce air pollution: catalysts, which are currently in use and constantly being improved upon; and nano-structured membranes, which are under development.

Catalysts can be used to enable a chemical reaction (which changes one type of molecule to another) at lower temperatures or make the reaction more effective. Nanotechnology can improve the performance and cost of catalysts used to transform vapours escaping from cars or industrial plants into harmless gasses. That's because catalysts made from nanoparticles have a greater surface area to interact with the reacting chemicals than catalysts made from larger particles. The larger surface area allows more chemicals to interact with the catalyst simultaneously, which makes the catalyst more effective.

Nanostructured membranes, on the other hand, are being developed to separate carbon dioxide from industrial plant exhaust streams. The plan is to create a method that can be implemented in any power plant without expensive retrofitting.

CLEANER WATER

Nanotechnology is being used to develop solutions to three very different problems in water quality. One challenge is the removal of industrial wastes, such as a cleaning solvent

called TCE, from groundwater. Nanoparticles can be used to convert the contaminating chemical through a chemical reaction to make it harmless. Studies have shown that this method can be used successfully to reach contaminates dispersed in underground ponds and at much lower cost than methods which require pumping the water out of the ground for treatment.

REDUCES WATER POLLUTION

Nanotechnology is being used to develop solutions to three very different problems in water quality. One challenge is the removal of industrial water pollution, such as a cleaning solvent called TCE, from ground water. Nanoparticles can be used to convert the contaminating chemical through a chemical reaction to make it harmless. Studies have shown that this method can be used successfully to reach contaminates dispersed in underground ponds and at much lower cost than methods which require pumping the water out of the ground for treatment.

The challenge is the removal of salt or metals from water. A deionization method using electrodes composed of nano-sized fibres shows promise for reducing the cost and energy requirements of turning salt water into drinking water.

The third problem concerns the fact that standard filters do not work on virus cells. A filter only a few nanometers in diameter is currently being developed that should be capable of removing virus cells from water.

CHEMICAL SENSORS

Nanotechnology can enable sensors to detect very small amounts of chemical vapours. Various types of detecting

elements, such as carbon nanotubes, zinc oxide nanowires or palladium nanoparticles can be used in nanotechnology-based sensors. Because of the small size of nanotubes, nanowires, or nanoparticles, a few gas molecules are sufficient to change the electrical properties of the sensing elements. This allows the detection of a very low concentration of chemical vapours.

IMPROVING CHEMICAL VAPOUR SENSORS

Nanotechnology can enable sensors to detect very small amounts of chemical vapours. Various types of detecting elements, such as carbon nanotubes, zinc oxide nanowires or palladium nanoparticles can be used in nanotechnology-based sensors. These detecting elements change their electrical characteristics, such as resistance or capacitance, when they absorb a gas molecule.

Because of the small size of nanotubes, nanowires, or nanoparticles, a few gas molecules are sufficient to change the electrical properties of the sensing elements. This allows the detection of a very low concentration of chemical vapours. The goal is to have small, inexpensive sensors that can sniff out chemicals just as dogs are used in airports to smell the vapours given off by explosives or drugs.

The capability of producing small, inexpensive sensors that can quickly identify a chemical vapour provides a kind of nano-bloodhound that doesn't need sleep or exercise which can be useful in a number of ways. An obvious application is to mount these sensors throughout an airport, or any facility with security concerns, to check for vapours given off by explosive devices.

These sensors can also be useful in industrial plants that use chemicals in manufacturing to detect the release of chemical vapours. When hydrogen fuel cells come into use, in cars or other applications, a sensor that detects escaped hydrogen could be very useful in warning of a leak. This technology should also make possible inexpensive networks of air quality monitoring stations to improve the tracking of air pollution sources.

NANOTECHNOLOGY APPLICATIONS UNDER DEVELOPMENT

Hydrogen sensor using a layer of closely spaced palladium nanoparticles that are formed by a beading action like water on a windshield. When hydrogen is absorbed the palladium nanoparticles swell, causing shorts between nanoparticles which lowers the resistance of the palladium layer.

Sensors using zinc oxide nano-wire detection elements capable of detecting a range of chemical vapours.

SPORTING GOODS

If you're a tennis or golf fan, you'll be glad to hear that even sporting goods has wandered into the nano realm. Current nanotechnology applications in the sports arena include increasing the strength of tennis racquets, filling any imperfections in club shaft materials and reducing the rate at which air leaks from tennis balls.

SPORTING GOODS WITH NANOTECHNOLOGY

FABRIC

Making composite fabric with nano-sized particles or fibres allows improvement of fabric properties without a significant

increase in weight, thickness, or stiffness as might have been the case with previously-used techniques.

HOW CAN NANOTECHNOLOGY IMPROVE FABRIC

Making composite fabric with nano-sized particles or fibres allows improvement of fabric properties without a significant increase in weight, thickness, or stiffness as might have been the case with previously-used techniques. For example incorporating nano-whiskers into fabric used to make pants produces a lightweight water and stain repellent material.

7

Rational Drug Discovery

In contrast to traditional methods of drug discovery which rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value. In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will have therapeutic value. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease states. The second is that the target is "drugable". This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule.

Once a suitable target has been identified, the target is normally cloned and expressed. The expressed target is then

used to establish a screening assay. In addition, the three-dimensional structure of the target may be determined.

The search for small molecules that bind to the target is begun by screening libraries of potential drug compounds. This may be done by using the screening assay (a "wet screen"). In addition, if the structure of the target is available, a virtual screen may be performed of candidate drugs. Ideally the candidate drug compounds should be "drug-like", that is they should possess properties that are predicted to lead to oral bioavailability, adequate chemical and metabolic stability, and minimal toxic effects. One way of estimating druglikeness is Lipinski's Rule of Five.

Several methods for predicting drug metabolism have been proposed in the scientific literature, and a recent example is SPORCalc. Due to the complexity of the drug design process, two terms of interest are still serendipity and bounded rationality. Those challenges are caused by the large chemical space describing potential new drugs without side-effects.

COMPUTER-ASSISTED DRUG DESIGN

Computer-assisted drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly.

Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. Semi-empirical, ab initio quantum chemistry methods, or density

functional theory are often used to provide optimized parameters for the molecular mechanics calculations and also provide an estimate of the electronic properties (electrostatic potential, polarizability, etc.) of the drug candidate which will influence binding affinity.

Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Alternatively knowledge based scoring function may be used to provide binding affinity estimates.

These methods use linear regression, machine learning, neural nets or other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target.

Ideally the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized. The reality however is that present computational methods provide at best only qualitative accurate estimates of affinity.

Therefore in practice it still takes several iterations of design, synthesis, and testing before an optimal molecule is discovered. On the other hand, computational methods have accelerated discovery by reducing the number of iterations required and in addition have often provided more novel small molecule structures.

Drug design with the help of computers may be used at any of the following stages of drug discovery:

- Hit identification using virtual screening (structure- or ligand-based design)
- Hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)

- Lead optimization optimization of other pharmaceutical properties while maintaining affinity

EXAMPLES

A particular example of rational drug design involves the use of three-dimensional information about biomolecules obtained from such techniques as x-ray crystallography and NMR spectroscopy.

This approach to drug discovery is sometimes referred to as structure-based drug design. The first unequivocal example of the application of structure-based drug design leading to an approved drug is the carbonic anhydrase inhibitor dorzolamide which was approved in 1995.

Another important case study in rational drug design is imatinib, a tyrosine kinase inhibitor designed specifically for the *bcr-abl* fusion protein that is characteristic for Philadelphia chromosome-positive leukemias (chronic myelogenous leukemia and occasionally acute lymphocytic leukemia). Imatinib is substantially different from previous drugs for cancer, as most agents of chemotherapy simply target rapidly dividing cells, not differentiating between cancer cells and other tissues.

Additional examples include:

- Many of the atypical antipsychotics
- Cimetidine, the prototypical H_2-receptor antagonist from which the later members of the class were developed
- Selective COX-2 inhibitor NSAIDs
- Dorzolamide, a carbonic anhydrase inhibitor used to treat glaucoma

- Enfuvirtide, a peptide HIV entry inhibitor
- Nonbenzodiazepines like zolpidem and zopiclone
- Probenecid
- SSRIs (selective serotonin reuptake inhibitors), a class of antidepressants
- Zanamivir, an antiviral drug
- Isentress, HIV Integrase inhibitor,

ANGIOTENSIN RECEPTOR BLOCKERS DRUG DISCOVERY AND DEVELOPMENT

The angiotensin receptor blockers (ARBs), also called angiotensin II receptor antagonists or sartans, are a group of anti-hypertensive drugs that act by blocking the effects of the hormone angiotensin II (Ang II) in the body, thereby lowering blood pressure. Their structure is similar to Ang II and they bind to Ang II receptors as inhibitors.Eg.,[T24 from Rhys Healthcare]

ARBs are widely used drugs in the clinical setting today, their main indications being mild to moderate hypertension, chronic heart failure, secondary stroke prevention and diabetic nephropathy.

The discovery and development of ARBs is a demonstrative example of modern rational drug design and how that design can be used to gain further knowledge of physiological systems, in this case, the characterization of the subtypes of Ang II receptors.

In 1898 physiologists Tigerstedt and Bergmann experimented with rabbits by injecting them with kidney extracts. Their results suggested that the kidneys produced a protein, which they named renin, that caused a rise in

blood pressure. In the 1930s, Goldblatt conducted experiments where he constricted the renal blood flow in dogs and he found that the ischaemic kidneys did in fact secrete a chemical that caused vasoconstriction.

In 1939 it was discovered that renin itself did not cause the rise in blood pressure but was an enzyme which catalyzed the formation of the substances that were responsible, namely, angiotensin I (Ang I) and Ang II.

In the 1970s scientists first observed that Ang II harms the heart and kidneys and it was also witnessed that individuals with high levels of renin activity in plasma were at increased risk of myocardial infarction and stroke.

With the introduction of angiotensin converting enzyme (ACE) inhibitors in the late 1970s it was confirmed that Ang II plays an important role in regulating blood pressure and electrolyte and fluid balance.

Before that attempts had been made to develop useful Ang II receptor antagonists and initially, the main focus was on angiotensin peptide analogues. Saralasin and other Ang II analogues were potent Ang II receptor blockers but the main problem was a lack of oral bioavailability.

In the early 1980s it was noted that a series of imidazole-5-acetic acid derivatives diminished blood pressure responses to Ang II in rats. Two compounds, S-8307 and S-8308, were later found to be highly specific and promising non-peptide Ang II receptor antagonists but using molecular modeling it was seen that their structures would have to mimic more closely the pharmacophore of Ang II.

Structural modifications were made and the orally active, potent and selective nonpeptide AT_1 receptor blocker losartan

was developed. In 1995 losartan was approved for clinical use in the United States and since then six additional ARBs have been approved. These drugs are known for their excellent side-effects profiles, which clinical trials have shown to be similar to that of placebos.

THE ANGIOTENSIN II RECEPTOR

The actions of Ang II are mediated by angiotensin receptors, AT_1 and AT_2. These receptors are members of the G protein-coupled receptors family which are seven transmembrane helices, connected by interchanging extracellular and intracellular loops.

Each G protein-coupled receptor couples to a specific G-protein which leads to activation of a special effector system. AT_1 receptors are for instance primarily coupled through the $G_{q/11}$ group of G-proteins.

Two more angiotensin receptors have been described, AT_3 and AT_4, but their role is still unknown.

DISTRIBUTION IN THE BODY

AT_1 receptors are mainly found in the heart, adrenal glands, brain, liver and kidneys. Their main role is to regulate blood pressure as well as fluid and electrolyte balance. AT_2 receptors are highly expressed in the developing fetus but they decline rapidly after birth. In the adult, AT_2 receptors are present only at low levels and are mostly found in the heart, adrenal glands, uterus, ovaries, kidneys and brain.

FUNCTIONS

Most of the known actions of Ang II are mediated through the AT_1 receptors, for example vasoconstriction, aldosterone

release, renal sodium reabsorption and vasopressin secretion. The AT_2 receptor also takes part in regulation of blood pressure and renal function but mediates antagonistic effects compared to the AT_1 receptor.

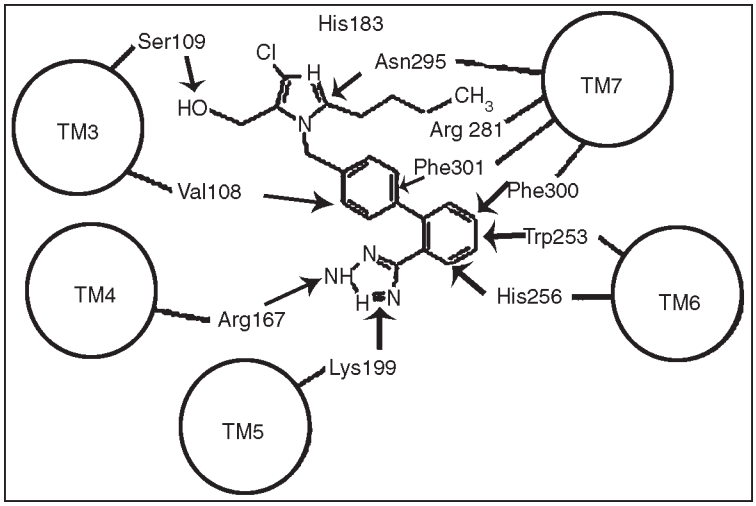


Fig. Losartan Receptor Binding

Ang II binds to AT_1 receptors via various binding sites. The primary binding site is at the extracellular region of the AT_1 receptor where Ang II interacts with residues in the N-terminus of the AT_1 receptor and its first and third extracellular loops.

The transmembrane helices also contribute to the binding via the C-terminal carboxyl group that interacts with Lys^{199} in the upper part of helix 5 of the receptor. The ionic bridge formed between Lys^{199} and the carboxyl terminal group of the Phe^{8} residue of Ang II is most likely stabilized by the Trp^{253} residue. In addition, Phe^{259} and Asp^{263} in transmembrane helix 6 and Lys^{102} and Ser^{105} in the outer region of transmembrane helix 3, have also been implicated in Ang II binding. This region may possibly participate in the stabilization of the receptor's ratification and in the formation of the intramembrane binding pocket.

RATIONAL DRUG DESIGN

Drug design, also sometimes referred to as rational drug design, is the inventive process of finding new medications based on the knowledge of the biological target. The drug is most commonly an organic small molecule which activates or inhibits the function of a biomolecule such as a protein which in turn results in a therapeutic benefit to the patient.

In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the biomolecular target to which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is often referred to as *computer-aided drug design*.

The phrase "'drug design" is to some extent a misnomer. What is really meant by drug design is ligand design. Modeling techniques for prediction of binding affinity are reasonably successful. However there are many other properties such as bioavailability, metabolic half life, lack of side effects, etc. that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to optimize using rational drug design techniques.

Typically a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen. Some approaches attempt to inhibit the functioning of the pathway in the diseased state by causing a key molecule to stop functioning.

Drugs may be designed that bind to the active region and inhibit this key molecule. Another approach may be to

enhance the normal pathway by promoting specific molecules in the normal pathways that may have been affected in the diseased state. In addition, these drugs should also be designed in such a way as not to affect any other important "off-target" molecules or antitargets that may be similar in appearance to the target molecule, since drug interactions with off-target molecules may lead to undesirable side effects. Sequence homology is often used to identify such risks.

Most commonly, drugs are organic small molecules produced through chemical synthesis, but biopolymer-based drugs (also known as biologics) produced through biological processes are becoming increasingly more common. In addition mRNA based gene silencing technologies may have therapeutic applications.

COMPUTER-AIDED DRUG REFINEMENT

It was at this time that the techniques of mass screening and combinatorial chemistry began to gain widespread acceptance and use.

The use of mass screening and combinatorial chemistry allowed researchers to discover lead compounds in a rapid and efficient manner. As such, *denovo* design tools and their associated problems were no longer needed to generate lead structures. One would surmise that computer-aided drug design technology would have soon ceased to exist. On the contrary, it soon became apparent that computational tools were needed that could optimize these lead compounds into potent drugs.

The concept of drug optimization versus *denovo* design is an important one. The difficulty with *denovo* ligand

generation is that an entire structure is being created from scratch. The confidence one has of accurately predicting how this structure will interact and bind within a target receptor is shaky at best. In drug optimization, we begin with a lead compound whose bound structure within the receptor has been characterized, most likely through x-ray crystallography.

Subtle modifications are then performed to generate derivative compounds using structure based drug design to improve binding affinity. Because we are making much smaller changes, our faith in the validity of the resulting structures is far greater. These derivatives then undergo testing to determine which modifications improve binding. The structures of the best ligands can then be elucidated to verify the accuracy of the modifications. This refinement process continues iteratively until optimal binding ligands are produced.

Since subtle modifications are being made to a common structure, the predictive ability of ligand refinement software is much higher. This is because the effect of a single chemical modification on ligand-receptor binding is far easier to quantitate than an extreme change. No longer are we trying to determine the binding affinities of drastically different structures. Instead, we are simply determining the rank order of a list of derivative compounds. This greatly increases the confidence that proposed structures will bind in a manner consistent with our understanding.

In addition, the act of generating chemical derivatives is highly amenable to computerized automation. Consider the application of targeted structure based combinatorial chemistry as discussed above. Libraries of derivative

components are assembled based upon the analysis of the active site. Because of the combinatorial nature of this method, an extremely large number of candidate structures may be possible.

A computer can rapidly generate and predict the binding of all potential derivatives, creating a list of the best potential candidates. In essence, the computer filters all weak-binding compounds, allowing the chemist to focus, synthesize, and test only the most promising ligands. Thus, utilizing computer aided drug design software to aid in the refinement of weak binding lead compounds is the most effective manner in which these tools can be employed. The use of computer modeling to refine structures has become standard practice in modern drug design.

PRINCIPLES DRUG FORMULATION

The formulation of a drug compound, *i.e.* the way in which is it combined with excipients (such as: binders, diluents, solubilizers and disintegrants) in, for example, tablets, capsules, creams pastes and gels can be critical to the performance of the active ingredient. The formulation must also be fully characterized to ensure product efficacy and patient safety. Techniques used for characterization include X-ray crystallography, vibrational spectroscopy, thermal analysis, solid state NMR and microscopy.

Polarized light microscopy, in particular, can be used to study a wide range of physicochemical properties. Materials can be characterized and identified by optical properties such as refractive index and birefringence and other phenomena such as particle size, particle shape, colour, twinning and the presence of inclusions or contaminants. When using a

heated stage further temperature related properties of materials may be investigated, such as polymorphic transitions (for example, crystallization and melting points).

During storage, it is essential that formulations are not susceptible to microbial contamination. Light microscopy can be used to determine the effectiveness of preservatives and antimicrobials. Many contaminants can be characterized using appropriate staining techniques.

BIOCHEMISTRY OF DRUGS

Enzymes are a subset of receptor-like proteins that are directly responsible for catalyzing the biochemical reactions that sustain life. For example, digestive enzymes act to break down the nutrients of our diet. DNA polymerase and related enzymes are crucial for cell division and replication. Enzymes are genetically programmed to be absolutely specific for their appropriate molecular targets. Any errors could have grave consequences. One can imagine the end result should blood clotting enzymes start activating throughout the body. Or consider the problems that arise when our immune system attacks our own tissues.

Enzymes ensure the specificity of their targets by forming a molecular environment that excludes interactions with inappropriate molecules. The analogy most often mentioned is that of a lock and key. The enzyme is a molecular lock, which contains a keyhole that exhibits a very specific and consistent size and shape. This molecular keyhole is termed the*active site* of the enzyme and allows interaction with only the appropriate molecular targets. Just as a typical lock is much bigger than the keyhole, the receptor is usually much

larger than the active site. The receptor, as specified by our DNA, is a folded protein whose major purpose is to form and maintain the size and shape of the active site.

The most important concept in drug design is to understand the methods by which the active site of a receptor selectively restricts the binding of inappropriate structures. Any potential molecule that can bind to a receptor is called a *ligand*. In order for a ligand to bind, it must contain a specific combination of atoms that presents the correct size, shape, and charge composition in order to bind and interact with the receptor. In essence, the ligand must possess the molecular key that binds the receptor lock. This is termed *steric complementarity*. As is the case with an actual key, if a different molecule varies by even a single atom in the wrong place, it may not fit properly, and will most likely not interact with the receptor. However, the more closely the fit between the ligand and receptor, the more tightly the interaction becomes. Again, keep in mind that this is only a two-dimensional schematic. Both ligand and active site are volumes with complex three-dimensional shape.

In addition to steric complementarity, electrostatic interactions influence ligand binding. Charged receptor atoms often surround the active site, imparting a localized charge is specific regions of the active site. From physics, we know that opposite charges attract while similar charges repel. *Electrostatic complementarity* further restricts the binding of inappropriate molecules since the ligand must contain correctly placed complementary charged atoms for interaction to occur. The main driving force for ligand and receptor binding is *hydrophobic interaction*. Nearly two-thirds of the

body is water, and this aqueous milieu surrounds all our cells. In order for ligand and receptor to interact, there must be a driving force that compels the ligand to leave the water and bind to the receptor. The hydrophobicity of a ligand is what causes this. Hydrophobicity stands for 'water fearing' and is a measure of how 'greasy' a compound is. It can be roughly approximated by the percentage of hydrogen and carbon in the molecule.

This force is easily demonstrated by placing a few drops of oil in a cup of water. The oil is composed of hydrocarbon chains and is highly hydrophobic. The oil droplets will instantly coalesce into a single globule in order to avoid the water, which is highly polar. Depending upon the size of the active site, there may be a myriad steric, electrostatic, and hydrophobic contacts. However, some are more important than others. The specific interactions that are crucial for ligand recognition and binding by the receptor are termed the *pharmacophore*. Usually, these are the interactions that directly factor into the structural integrity of a receptor or are involved in the mechanism of its action. Using our lock and key analogy from above, we can imagine a lock having numerous tumblers.

There may be many keys that can sterically complement the lock and fit within the keyhole. However, all but the correct key will displace the wrong tumblers, leading to a sub-optimal interaction with the lock. Only the correct key, which presents the pharmacophore to the receptor, contacts the appropriate tumblers and properly interacts with the lock to open it. This is crucial to the design of pharmaceuticals since any successful drug must incorporate the appropriate chemical

structures and present the pharmacophore to the receptor.

In the upper left frame of this figure, we see our native ligand bound within the active site. Assume that through biochemical investigation, we determine that the phenyl ring and the carboxylic acid group are vital to receptor interaction. Thus, we deduce that these two groups must be the pharmacophore that a ligand must present to the receptor for binding. In future drugs that we develop to mimic the native ligand, we must include these two pharmacophoric elements for successful binding to occur.

This is shown in the upper right derivative compound where a bicyclic group has been substituted. Because it maintains the pharmacophore and retains its complementary size and shape, it has a reasonable chance of successfully binding. However, any drug that we develop which lacks a complete pharmacophore may not interact withz the receptor target.

When a medical condition exists where a drug could be beneficial, extensive scientific study must first be done in order to determine the biological and biochemical problems that underlie the disease process. This often takes years of study in order to characterize the targets for a potential drug. The reason is that nearly all biological processes in the human body are tightly interconnected. Altering the behaviour of select receptors or enzymes may have detrimental effects with other systems.

These are the side effects that occur with nearly all drugs. Furthermore, the human body is a homeostatic machine, and always attempts to achieve equilibrium. As a result, the body will attempt to counteract any pharmacotherapeutic

intervention. Once a receptor target has been established and well characterized, the process of ligand design begins. Obviously, the first consideration is that the designed ligand must complement the active site of the receptor target. Steric, electrostatic, and hydrophobic complementarity must be established as we discussed above. The pharmacophore must be presented to the receptor in order for recognition and binding to occur.

In addition to adequately binding the receptor, the biochemical mechanism of the receptor target must be taken into consideration. If the peptide bond is present at a specific position in the active site when the ligand binds, it is cleaved by the protease with the addition of water (H_2O) to form two separate fragments. If our goal is to inactivate this protease, any designed ligand cannot possess this peptide bond at the same position.

Otherwise, it will simply be cleaved by the protease, and the protease will continue to function unperturbed. However, the ligand can be modified so that the peptide bond is no longer present as shown in the bottom portion of the figure. If the enzyme then binds this ligand, the enzyme will not be able to cleave it. As such, the enzyme would be inactivated, as the ligand remains lodged in the active site.

Having characterized the active site region and the mechanism of action of the target receptor, the challenge then becomes one of designing a suitable ligand. This is, by far, the most daunting task of the entire drug design process. The optimal combination of atoms and functional groups to complement the receptor is often the natural ligand of the receptor. Unfortunately, this is usually an unacceptable

candidate for a drug. This is because the natural ligand either fails to inactivate the receptor, as described above, or it is a natural substance that cannot be patented. Patent considerations are often paramount, as legal protection for the developed drug affords the opportunity to recoup the financial costs of development. Therefore, alternate combinations of chemical structures must be devised.

The design of novel ligands is often restricted by what chemists are physically able to synthesize. It is of no use to design the ultimate drug if it cannot be manufactured. The laws of chemistry dictate that each atom type has a specific size, charge, and geometry with respect to the number and types of neighbouring atoms that it can be joined to. The entire field of chemistry is predicated on the establishment of synthetic rules for the construction and manipulation of various combinations of atoms and functional groups. It is the expertise in these chemical rules that govern the ability of the synthetic chemist to design and synthesize postulated ligand candidates. Within these rules, the drug developer must creatively propose suitable chemical structures that satisfy the requirements discussed above.

Finally, there are biological considerations to the development of new drugs. The liver is the major organ of detoxification in the human body. Any drug that is taken undergoes a number of chemical reactions in the liver as the body attempts to neutralize foreign substances. This set of reactions is well characterized, and a great deal of knowledge exists as to how drugs are modified as the body eliminates them. More importantly, various chemical structures are highly toxic to biological systems, and these are also well

characterized. These constraints must also be taken under consideration as novel drugs are developed.

NANOTECHNOLOGY IN DRUG DISCOVERY

The result is the determination of specific receptor targets that must be modulated to alter their activity in some way. Once these targets have been identified, the goal is then to find compounds that will interact with the receptors in some fashion. At this initial stage of drug development, it does not matter what effect the compounds have on the targets. We simply wish to find anything that binds to the receptor in any fashion.

The first step is to determine an *assay* for the receptor. An assay is a chemical or biological test that turns positive when a suitable binding agent interacts with the receptor. Usually, this test is some form of colorimetric assay, in which an indicator turns a specific colour when complementary ligands are present.

This assay is then used in *mass screening*, which is a technique whereby hundreds of thousands of compounds can be tested in a matter of days to weeks. A pharmaceutical company will first screen their entire corporate database of known compounds. The reason is that if a successful match is found, the database compound is usually very well characterized. Furthermore, synthetic methods will be known for this compound, and patent protection is often present. This enables the company to rapidly prototype a candidate ligand whose chemistry is well known and within the intellectual property of the company.

If a successful match is found, the initial hit is called a *lead compound*. The lead compound is usually a weakly binding ligand with minimal receptor activity. The binding of this structure to the receptor is then studied to determine the interactions that foster the ligand-receptor association. If the receptor is water soluble, there is a chance that *x-ray crystallographic analysis* can be employed to determine the three-dimensional structure of the ligand bound to the receptor at the atomic level.

This is a very powerful tool for it allows scientists to directly visualize a snapshot of the individual atoms of the ligand as they reside within the receptor. This snapshot is referred to as a *crystal structure* of the ligand-receptor complex. Unfortunately, not all complexes can be analysed in this manner.

However, if a crystal structure can be determined, a strategy can then be developed based upon this characterization to improve and optimize the binding of the lead compound. From this point onward, a cycle of iterative chemical refinement and testing continues until a drug is developed that undergoes clinical trials. The techniques most often used to refine drugs are *combinatorial chemistry* and *structure based design*.

Combinatorial chemistry is a very powerful technique that chemists can employ to aid in the refinement of the lead compound. Combinatorial chemistry is a synthetic tool that enables chemists to rapidly generate thousands of lead compound derivatives for testing. As shown above in Figure, a scaffold is employed that contains a portion of the ligand that remains constant.

Subsite groups (shown in red, green, and blue) are potential sites for derivatization. These subsites are then reacted with combinatorial libraries to generate a multitude of derivative structures, each with different substituent groups. One can see how a vast number of compounds can be generated as a result of the combinatorial process. If a scaffold contains three derivatization sites and the library contains ten groups per site, theoretically 1000 different combinations are possible.

Structure based design, often called rational drug design, is much more focused than combinatorial chemistry. As shown above in Figure above, it involves using the biochemical laws of ligand-receptor association discussed above to postulate ligand refinements to improve binding. For example, we discussed that steric complementarity is vital to tight receptor binding.

Using the crystal structure of the complex, we can target regions of the ligand that fit poorly within the active site and postulate chemical changes to improve complementarity with the receptor. In a similar fashion, functional groups on the ligand can be changed in order to augment electrostatic complementarity with the receptor. However, the danger in altering any portion of the ligand is the effect on the remaining ligand structures. Modifying even a single atom in the middle of the ligand can drastically change the shape of the overall structure. Even though complementarity in one portion of the ligand might be improved by the chemical revision, the overall binding might be severely compromised. This is the difficulty in any ligand refinement procedure.

8

Synthetic Drugs

The term "Synthetic," as used in the National Drug Control Strategy (NDCS) Annual Report and the National Synthetic Drugs Action Plan, refers to drugs whose origins are not primarily organic, but rather are produced via chemical synthesis. For clarification, heroin, cocaine, and marijuana are in the organic category, while LSD, MDMA, and methamphetamine are synthetics. This report deals primarily with amphetamine-type stimulants often termed "designer drugs" or "club drugs " and drugs such as oxycodone or benzodiazepine, which are produced commercially by drug manufacturers for valid medical purposes, and are diverted from legal channels or produced illegally in clandestine laboratories for illicit markets worldwide. Regardless of source, commercial or clandestine, amphetamine-type stimulants (ATS) are emerging as a class of drugs that are widely abused in the United States and pose a serious threat to our youth. Some of the most prevalent synthetic drugs of abuse are:

METHAMPHETAMINE

Methamphetamine is a central nervous system stimulant with serious health implications that include violent behaviour, extreme paranoia, and other psychotic episodes. The drug is clandestinely manufactured in crude laboratories primarily located in Mexico and the United States by street chemists referred to as "cooks." Usually injected or snorted, the drug can also be smoked or ingested orally. The manufacture of methamphetamine poses serious environmental hazards.

3-4 METHYLENEDIOXYMETHAMPHETAMINE (MDMA)

Commonly referred to as Ecstasy or XTC, MDMA is a psychoactive substance with both stimulant and mild hallucinogenic properties. Most often found in tablet form, although occasionally distributed as a crystalline powder. Taken orally, the health risks include severe hyperthermia, dehydration, and long term learning impairment. MDMA is manufactured in laboratories primarily located in Western Europe at this time.

PARAMETHOXYAMPHETAMINE (PMA)

PMA is a tablet form amphetamine derivative similar to MDMA (Ecstasy) but more lethal even in smaller doses. More than 50 milligrams may be fatal. PMA producers sometimes use the same imprinted logos on PMA that are used on MDMA tablets in order to market the product as MDMA to users, resulting in fatalities from overdose or mixing of the drug with MDMA.

GAMMA HYDROXYBUTYRATE (GHB)

GHB is a central nervous system depressant usually sold as an odorless, colorless liquid in spring water bottles or as

a powder mixed with beverages and soft drinks, reportedly used in date-rapes.

GAMMA BUTYROLACTONE (GBL)

Often found in industrial cleaners, GBL is the precursor chemical for the manufacture of GHB. In addition, it has been marketed as a nutritional supplement in health food stores and over the Internet in both powder and capsule form. GBL is synthesized by the body to produce GHB. Ingestion of GBL often causes a severe physical reaction, usually through the violent regurgitation of the fluid. These chemicals increase the effects of alcohol, and can cause respiratory distress, seizures, coma, and death.

KETAMINE

A prescription general anesthetic with some physical effects similar to PCP and visual effects of LSD, ketamine is primarily marketed for veterinary use. Sold as both a liquid and a powder, use in humans can cause delirium, amnesia, depression, long-term memory and cognitive difficulties. Due to its disassociative effects, ketamine is reportedly used as a date-rape drug.

ROHYPNOL

Rohypnol, a sleep aid that has never been manufactured or marketed in the United States, it is still produced in Mexico and Europe and available by prescription in many countries. It is smuggled into the United States from Mexico. Capable of producing extreme lethargy and significantly reducing recall capability of the brain; it has been often mentioned in relation to numerous date rapes (though the

data do not support the contention that it is widely used for this purpose). Abuse of rohypnol is generally episodic use among teenagers and young adults as an "alcohol extender" and disinhibitory agent, most often in combination with beer.

LYSERGIC ACID DIETHYLAMIDE (LSD)

LSD is an extreme hallucinogen most commonly marketed on blotter paper or in gelcap form. It has been known since the 1960's for its hallucinogenic properties and the adverse psychotic side effects that frequently occur during and after use (flashbacks). It is a clandestinely manufactured crystalline powder reduced to a clear liquid for dosing purposes. Generally it is produced by rogue chemists, some with extensive academic backgrounds in chemical synthesis, in relatively professional laboratory surroundings. Chemistry background, laboratory control knowledge, and an educated understanding of the process is generally required to successfully and safely conduct the synthesis.

PHENCYCLIDINE (PCP)

Commonly known as "angel dust," PCP was originally developed as an animal tranquilizer. It is now clandestinely manufactured in environments known as "bucket labs" by street chemists. In both a powder and liquid form, it is almost always smoked by placing it on a marijuana cigarette or regular menthol cigarette, known as a "kool-dip." It is an extremely dangerous substance causing coma, convulsions, and psychotic delusions. Users are known to become extremely violent and aggressive, commonly causing injuries to themselves and others.

OXYCONTIN

OxyContin is a synthetic, opiate, prescription pain medicine often used in the treatment of pain related to cancer and other debilitating conditions. OxyContin contains the drug oxycodone, which is a common drug used in pain relievers such as Percocet and Percodan. However unlike other forms of oxycodone, OxyContin is available in higher dose units, as a time-release formulation. Law enforcement sources have reported an increase in the diversion of OxyContin and other medications containing oxycodone. This increase in illegal use has been especially apparent on the East Coast. The increase in the abuse of OxyContin has led to an increased number of pharmacy robberies and health care fraud incidents.

PRECURSOR CHEMICALS

Necessary precursors, despite efforts to restrict them, are widely available worldwide. Thirty-four chemicals are listed by federal statute, Section 812 of the Controlled Substances Act, as controlled substances on the Drug Enforcement Agency's (DEA) controlled substance schedules.

ORGANIC IMPURITIES IN CHEMICAL DRUG SUBSTANCES

SUBSTANCES

Many pharmacologically active substances are totally synthetic organic chemicals, which are produced in bulk quantities by the active pharmaceutical ingredients (APIs) manufacturers to comply with good manufacturing practices

(GMPs). Some are also highly purified and well-characterized, naturally occurring active substances. Chemical purity of a synthetic API, a characteristic with a significant impact on the drug product quality, is accomplished only if impurities are each present at a nominal concentration less than or equal to a predefined limit.

Impurities are unwanted coexisting components in bulk pharmaceutical chemicals that arise during manufacture and/or subsequent storage. According to the definition given by the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, impurity is any component of a substance for pharmaceutical use that is not the chemical entity defined as the substance. Excluding enantiomers, polymorphic forms and extraneous contaminants — the presence of the last is inconsistent with GMP — ICH classifies impurities associated with a chemical API as inorganic, organic (process- and drug-related) and residual solvents.

However, some medicinal substances are mixtures of closely related compounds. These compounds have similar activity; they contribute to the assay result and are not regarded as impurities (such an example is cefamandole free acid in the pure form of cefamandole nafate). Budesonide epimer A is not regarded as an impurity although it must be controlled to ensure batch-to-batch consistency and uniformity among different manufacturers. Wherever a substance is supplied in pure form as an organic or inorganic salt, the organic acid or the inorganic counter ion are not considered impurities (examples include maleic acid in enalapril maleate, benzene sulfonate in amlodipine besilate

and chlorides in substances supplied as hydrochloride salts). Coexisting water in pure APIs is not an impurity either.

ORGANIC IMPURITIES IN SYNTHETIC APIS

ICH further classifies organic impurities as starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts. Enantiomers, which are not included in the previous classifications, are stereoisomers with the same molecular formula as the drug substance, differing only in the spatial arrangement of atoms within the molecule and having a nonsuperimposable mirror image relation (chirality). Enantiomers have the same physical and — in an achiral environment — chemical properties, except the optical rotation.

Diastereoisomers (isomers of drugs with more than one chiral centre) and geometric isomers are both chemically distinct, pharmacologically different and readily separated.

Many of the marketed drugs are chiral and are often supplied as mixtures of enantiomers (racemates) rather than single enantiomers.

However, certain single enantiomeric forms of chiral drugs are regarded as improved chemical entities with a better pharmacological profile. Some of the chiral drug substances offered as pure enantiomers are naturally biosynthetic products. In these cases, the presence of the enantiomeric impurities is excluded because of the high level of enantioselectivity of their biosyntheses.

However, technological advances such as chiral separation or assymetric synthesis permit commercial production of several single enantiomer drugs. In the manufacture and control of these drugs the other enantiomer

(antipode) is an undesirable organic impurity.[3] For this reason, chiral chromatographic tests are included in the pharmacopoeial monographs of some single enantiomeric drugs. In the future such tests will become more common. A presentation somewhat different from that of ICH, but very detailed, which also gives useful information on the chemical and analytical characterization of the related organic impurities in drug substances and drug products, has recently been published.

Each impurity must be investigated with respect to both chemistry and safety aspects. The former include identification (structural characterization), reporting and quantitation using suitable analytical procedures, while the latter include a process of acquiring and evaluating data concerning the biological safety of an impurity (qualification). Individually listed impurities, limited with specific acceptance criteria, are referred to as specified and they can be either identified or unidentified.

Unspecified impurities are limited by a general acceptance criterion. A decision tree for the identification and qualification along with the corresponding thresholds, which are dependent on the maximum permitted daily dose (MDD), is given by ICH.

Summing up, the following list of organic impurities must be presented in the specification of a synthetic drug substance:

- Each specified identified or unidentified impurity
- Any unspecified impurity
- Total impurities.

Specified unidentified impurities are referred to by an

appropriate qualitative analytical description (*e.g.*, relative retention time).

CONTROL OF ORGANIC IMPURITIES

A description of the identified and unidentified existing impurities in a chemical drug substance is referred to as the impurity profile (IP).

Impurity profiling includes the procedure aimed at the detection, structure elucidation/identification and the quantitative determination of these impurities. Efforts are mainly focussed on the profiling of the organic impurities as the other possible groups, such as inorganic impurities and residual solvents, are easily identified and their toxicity is known. The presence of organic impurities in a drug substance is closely dependent on the process of manufacture. A different route of synthesis will tend to lead to a different IP.

In pharmaceutical research and development, IP is often decided by using high performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry. Direct coupling or multiple hyphenation of these techniques along with the use of modern software for spectral/ chromatographic searching is a valuable tool for the detection of impurities at trace levels. In case of volatile, but thermally stable compounds gas chromatography (GC) coupled with various detection systems still plays an important role. Investigation of the impurities in complex natural products by using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS has been proposed. Capillary electrophoresis and solid phase

microextraction/GC–MS have also been successfully used. Normally, more than one analytical system is applied for the confirmation of an IP.

Isocratic and gradient reversed-phase HPLC with ultraviolet-visible (UV-Vis) detection remains the most suitable analytical procedure for routine impurity testing. Baseline separation of all the potential organic impurities and the active substance should be performed. Better specificity is established by using photodiode array detectors, when the method is under development. In certain applications ion pairing offers better peak separation and post-column derivatization lowers detection limits. GC and thin layer chromatography (TLC) are often applied in the industrial quality control (QC) laboratories for impurity testing. TLC determinations have a semi-quantitative nature, but allow the detection of impurities completely retained or those not retained at all by the stationary phase.

PHARMACOPOEIAL STATUS

The quality of a chemical active substance with respect to organic impurities is controlled by a set of tests within a pharmacopoeial monograph. Individual monographs are periodically updated to keep pace with scientific progress and regulatory developments. Following the revised ICH Q3A impurity testing guideline major pharmacopoeias will continue publishing new or revised relevant monographs and general chapters. Active substances found to contain an organic impurity not detected by the relevant pharmacopoeial tests prescribed below are not of pharmacopoeial quality, unless the amount and the nature of this impurity is

compatible with GMP. Two general chapters of the *US Pharmacopeia* (*USP*) deal with organic impurity testing. Concepts and definitions are clearly described although a somewhat different terminology from that of ICH is used.

Until now, one of three types of tests in bulk pharmaceutical chemicals is ordered:

1. A chromatographic purity test coupled with a non-specific assay
2. A chromatographic purity-indicating method that also serves as an assay
3. A specific test and limits for known impurities, a procedure that requires reference standards for these impurities.

In the future, new and revised *USP* individual monographs will include tests that actually control specified and unspecified organic impurities. Where different routes of synthesis yield different IPs, perhaps different analytical procedures will be proposed. All specified impurities will be separately limited, with a further limit of 0.10% for any unspecified (unknown) impurity. Total impurities above the disregard limit should be less than 1.0%.[11] *USP* also proposes that a suitable test for detecting impurities that may have been introduced from extraneous sources, should be employed in addition to tests provided in a specific monograph.

The European Commission decided that the principles and terminology of the revised ICH Q3A should be implemented in the *European Pharmacopoeia* (*EP*) monographs of the active substances, both new and already published. A new general chapter concerning the control of

impurities in pharmaceutical substances was introduced in the fifth edition of the *EP*, while a revision of the monograph entitled *Substances for Pharmaceutical Use* has also been done. According to the policy of *EP* control of the relevant organic impurities in synthetic drug substances is often accomplished by the test of related substances. Currently, it is a limit test (comparison of the peak areas), but will progressively be changed to utilize a quantitative acceptance criterion.

Some individual monographs already satisfy this demand. More tests are ordered, if the general test does not control a given impurity or there are other special reasons. Potential impurities with a defined structure that are known to be detected by the tests in a monograph, but are not known to be present in medicinal substances above the identification threshold, are referred to as detectable impurities. They are limited by a general acceptance criterion. *EP* individual monographs published in the new format include a separate section in which all impurities (specified and detected) are listed. Unidentified specified impurities are not listed in this section, but their specific acceptance criteria along with appropriate analytical characteristics (*e.g.*, retention time) are reported in the text, wherever it is applicable.

However, previous *EP* monographs not having a related substances test in the new explicit style are to be read and interpreted according to the recent amendments. During the coming years, *EP*individual monographs now published in the old format will be revised to contain related substances tests and lists on specified and other detectable impurities. Monographs containing tests for related substances based

on TLC will also be revised. *EP* specifications for the coexisting organic-related impurities in selected drug substances are summarized in Table 1. The maximum daily dose (MDD) of all the substances presented therein is d"2.0 g. Acceptance criteria, as shown, are to be tabulated after having interpreted the information given in each separate monograph in conjunction with the general chapters mentioned.

Clarithromycin, a semisynthetic macrolide antibiotic, is also included in this table. Total clarithromycin impurities are limited to a nominal concentration of less than or equal to 3.5%. In certain *EP*-specific monographs related substances tests cover different IPs. In these cases only impurities for the known profile from a single source need to be reported in the specifications shown in the certificate of analysis (CoA), unless the same master form of CoA is issued for a specific active substance produced by more than one chemical pathway and thus having different IPs.

Pharmacopoeial chromatographic purity tests must be routinely checked for correct performance. Identification of peaks must not be based on absolute terms (retention time), as these are system dependent. Relative retention times for each named impurity are usually quoted. Reference substances for peak identification are offered in certain applications. The chromatograms obtained with the solutions prepared from these reference materials are used for the correct peak identification and for system suitability testing too.

The quantification of an impurity, wherever possible, is performed by using a solution of the reference substance of

the impurity. Alternatively, a reference solution of the substance being examined, containing a known amount of the impurity, may be used. Using either of the procedures the result obtained expresses a real percentage of the impurity in the sample. When neither of these procedures is possible and the impurity in question has a response similar to that of the substance under examination, a dilution of the solution of the substance (the test solution or a solution made of the reference substance) is used as a reference solution. The result so obtained expresses a nominal percentage of the impurity in the sample. If a named impurity is known to have a significantly different response a correction factor is used for the calculation of the content of this impurity in the substance being examined.

The correction factor is the reciprocal of the response factor. For the calculation of the nominal percentage of the impurity in the substance being examined, the area of the peak because of the impurity in question in the chromatogram obtained with the test solution must be multiplied by this factor. The peak to which the factor is applied must be identified unambiguously. In all approaches described earlier numerical results should be reported to two significant figures. Conventional rules should be used for rounding.