Drug Discovery

A History

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The quest for the elixir of life by the mediaeval alchemists may not have resulted in success, but the last one hundred years have witnessed the introduction of a number of drugs that have literally saved countless millions of lives. Society at large is indebted to the men and women in the laboratories and clinics of the pharmaceutical industry, research institutes, hospitals and universities whose commitment to science has brought about what in an earlier era would simply have been called miracles. This book is dedicated to these men and women.
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Preface

The contents of this book range from the faltering attempts of our ancient ancestors to discover herbal remedies to the present quest of scientists to develop safe, efficacious medicines. This will enable readers to understand both why efficacious drugs were not developed until the twentieth century and why progress has been so rapid over the past fifty years.

The book consists of three parts. The first part progresses from the possible use of medicinal herbs by Neanderthals on to the endeavours of ancient civilisations in Mesopotamia, Egypt, Greece and Rome. A subsequent consideration of the impact of Greek medicine on the Arab world and then renaissance Europe is followed by an examination of the heritage of the alchemists whose attempts to transmute base metals into gold provided the chemical techniques that allowed purified metallic compounds to be introduced into medicine. After reviewing how the influence of the enlightenment on drug discovery was impeded by the introduction of unsubstantiated medical systems that were popular from the seventeenth to nineteenth centuries, this part of the book concludes with an account of the twentieth century development of life-saving organic compounds incorporating various metals.

The second part of the book examines the commonest source of drug prototypes from which other medicines are derived – the natural world. Prototypes from vegetable, animal and microbial sources are described in separate chapters, as are the medicinal compounds prepared from them. The problem of relying on nature as a source of drug prototypes is confronted.

Modern drug discovery has consisted of a series of thematic developments that began with the isolation of pure alkaloids and glycosides from plants in the early years of the nineteenth century. One hundred years later, a parallel development witnessed the isolation of pure hormones from mammalian sources. The chapters throughout the book are organised so as to heighten awareness of such thematic developments, but this is particularly relevant in the second part of the book.

The third part considers those synthetic compounds that have served as drug prototypes, followed by a consideration of compounds derived from them. The role of serendipity in providing synthetic prototypes is also investigated. The book ends with a cautionary note concerning the future of drug discovery.

The specialist reader will find that the book contains an extensive list of bibliographic references relating to the discovery of drugs, but the book has been written for the general reader. No particular expertise in chemistry is assumed, but chemical structures have been interspersed with the text to enable those familiar with the subject to obtain a deeper understanding. Their presence should also enable others to begin to appreciate why chemistry has appealed to so many great minds. The chemical structures are presented in such a way as to allow even those with little experience of the subject to understand how prototypes and compounds obtained from them were manipulated in order to provide novel compounds with unprecedented healing properties.

Walter Sneader
December 2004
One of the best presents I ever had was a copy of Dr Sneader’s 1985 book Drug Discovery: The Evolution of Modern Medicines. It was a great introduction to the fascinating world of the way in which many medicines have been discovered, a world of which I had gained only snippets from books on clinical pharmacology. And now Dr Sneader has produced not just an updated version of that book but a completely new work which comprehensively spans the whole subject from the earliest times to the present.

Dr Sneader is exceptionally well qualified to write such a book having trained in both pharmacy and medicinal chemistry. His distinguished academic career at the University of Strathclyde, Glasgow, where he is the Head of the School of Pharmacy, includes the writing of two other acclaimed books, Drug Development from Laboratory to Clinic and Drug Prototypes and Their Exploitation.

The author takes us right back to the pre-historic period, but he does so critically and with caution as to how to interpret earlier evidence, for example as to when opium poppy was first used. Critical evaluation is a hallmark of Dr Sneader’s writing. I like the way that the book has been divided into three major sections, dealing first with previous history, then with drugs developed from natural compounds and finally with synthetic drugs. Phytochemicals are of especial interest and we know that over 30 modern medicines owe their origin to plants in one way or another. Sometimes a folk remedy has stood by itself, but more often it has been scientific investigation that has either found a new use for the plant substance, or has developed a synthetic compound from it. Here as elsewhere one is continually impressed by the details that the author gives as to who were the actual people making the discoveries. Likewise Dr Sneader has a plethora of fascinating stories, for example the one that recounts how the discovery of coal gas for illuminating the home led to the use of antiseptic treatment by Joseph Lister. In another he relates how the Nazis suppressed the fact that aspirin was developed by a Jewish chemist.

Natural remedies or their derivatives are of great importance but so are purely synthetic drugs, sometimes sneered at by the ignorant as “chemical medicine”, not realising that chemical molecules are the same whether produced by nature or by a pharmaceutical company. The beta blockers owed everything to Sir James Black’s ideas and nothing to the natural world.

Dr Sneader deals with synthetic drugs in one complete section of the book and here as elsewhere our understanding is greatly helped by the chemical formulae he carefully places within the text. This is where his expertise in chemistry is so useful.

A good chapter is the one on screening. Companies are doing mass screening of natural compounds in the hope of finding an interesting looking molecule, and furthermore by combinatorial chemistry they are creating huge numbers of totally new compounds. The author notes that there are 17 million compounds on the chemical register, but will screening find a new treatment for say psoriasis?

It is a pleasure to give a whole hearted commendation to a really fine account of a very important subject, written and written clearly by someone who has a deep understanding of it. There are many references to the literature and the index is excellent.

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The quest to discover healing drugs has always been influenced by prevailing social and cultural factors, one of the most important being the ability to communicate what has been learned to others. The success of modern drug research has in no small measure been dependent upon the rapid and universal publication of scientific results. Before the appearance of writing about five thousand years ago, accumulated knowledge and wisdom could only be passed on by word of mouth from generation to generation, or be preserved in epics and poems recited by bards who memorised their content. In some instances, these verses were eventually committed to writing.

According to some, ancient paintings on the walls of caves may hold clues to the early use of drugs that had a pronounced effect on the mind. However, it is only the written accounts that communicate something of the reasoning that led our ancestors to seek out remedies for treating disease, such as those compiled by the inhabitants of Ancient Egypt, Mesopotamia and particularly Greece.

How our earliest ancestors selected drugs is a matter for speculation. Clues may be found by considering the behaviour of contemporary primitive peoples and the nature of their folk medicines. This, in conjunction with the written record, suggests that most societies initially adopted a straightforward empirical approach to treatment of the minor ailments of everyday life, selecting healing herbs by a process of trial and error. Those living in hot, sandy desert conditions sought out soothing balms and lotions to apply to their dry skin and eyes, while others whose diet was deficient in fibre sought herbs to relieve their constipation. The ache following the eating of contaminated foods was relieved by swallowing other herbs that irritated the wall of the stomach to induce vomiting, while the bleeding of minor cuts or the pain of burns was dealt with by rubbing with leaves or barks rich in astringent tannins.

Serious illness was a different matter. Unable to explain how it was possible to be fit and healthy one moment yet be writhing in agony or even dying a moment later, our ancestors turned to magic and the supernatural. The art of healing became inextricably interwoven with magic and religion. Disease was then seen as a manifestation of the power of demonic characters to enter the body, presumably through one of its orifices. Attempts were made to expel these demons through their supposed route of entry by administering drugs that could produce vomiting, purgation, urination or sneezing. An alternative was to administer foul-tasting or obnoxious substances such as dung or dead flies. Sympathetic magic was also employed, whereby properties associated with plants, animals or other objects were deemed to be transferable to the sick. A patient might then have been treated with a skin newly sloughed from a reptile because the act of sloughing was considered to be a process of life renewal. Whether expurgating demons or transferring life-endowing properties, the objective was to treat the demon-possessed patient rather than a specific disease. Therein lies the origins of holistic medicine.

Witch doctors, sorcerers, shamans and medicine men preceded their administration of herbs by elaborate rituals involving fervent dancing, weird utterances, grotesque facial expressions, use of charms and other devices – all likely to have a powerful effect on the mind of the sick.
person and thereby enhance any benefit to be derived from the treatment. Even when belief in
magic was undermined by the arrival of both pantheistic and monotheistic religions, the
remedies themselves hardly changed. Treatment was instead administered in conjunction with
appropriate benedictions rather than the incantations previously uttered in the hope of driving
out the demons. The successes seen in those few patients who recovered despite the
ministrations of their attendants were probably produced by the incantations and benedictions
rather than the medicaments themselves, for these were invariably naturally occurring
substances of which few could produce any effect other than purgation. Failure was explained
away by asserting that the medicament had not been properly prepared or administered.

Once a truly effective remedy has been recognised, the quest for alternatives usually dissipates
or, in our modern era, becomes limited to a few commercially viable alternatives. It is a telling
point that the natural products employed in the past, and still favoured in some quarters, were
frequently recommended for a wide variety of disparate medical conditions, a feature that is
suggestive of a lack of any clearly defined effect. This is not to say that none of the ancient
remedies had any beneficial action; the few that did were the exception rather than the rule.

When the philosophically inclined Greeks arrived on the scene, supernatural beliefs were
rejected in favour of rational concepts. The quest to find drugs became based on the belief that
they had to correct an imbalance in body humours thought to be the cause of disease. This was
to have a major impact on the selection of plants for use as drugs.

The writings of the second century physician Galen so convincingly presented the
accumulated knowledge of Greek medicine that they dominated medical thinking until the
seventeenth century or later. During the Dark Ages, however, medical knowledge became
fossilised in much of Europe. The asceticism of the Christian Church fostered the attitude that
treating the sick was in the hands of the Almighty alone. Eventually, it was recognised that the
soul had still to be cared for and consequently some monasteries became hospitals. These
acquired surviving medical manuscripts which monks laboriously copied by hand. Herbal
gardens were also established to supply medicines, yet new treatments were not introduced, or
if they were then it was in the hands of local folk healers who did not record their observations
for posterity.

The Christian influence did not extend to the Arab world. There, ancient texts were
translated and given a new lease of life, especially in ninth century Baghdad. Arab physicians
took delight in the legacy from their Greek counterparts and compiled treatises that
elaborated at length on this and their own contributions to therapeutics. It was from the Arab
world that Europe was to rediscover its own medical heritage three centuries later, when the
first European medical school was established in the southern Italian port of Salerno. Its
influence and that of its successors quickly developed, largely because the Church took steps to
prevent the practice of medicine in its monasteries out of concern that it was in danger of
becoming their prime activity.

The secularisation of medicine in Europe helped its practitioners to question the
authoritarianism that had crippled it for so long. Another factor in this was the introduction
of the printing press in the second half of the fifteenth century, which gradually made available
the old Greek herbals and the Arab additions to them. By this time the Renaissance was well
under way and there were those prepared to challenge the authoritarian views of Galen and his
devotee ibn Sina. Prominent among them was the maverick physician Paracelsus. His writings
were complex and at times obscure, but they attracted a band of followers who were to change
drug therapy. Among them were chemists who strove to isolate active principles from
medicinal preparations rather than attempt to transmute base metals into gold as the
alchemists had aspired. Initially, they met with some success when using traditional alchemical
techniques such as distillation or sublimation to refine metallic compounds. However, when
plants were exposed to these techniques, the heat treatment involved destroyed any active
principles. All that could be obtained were odiferous waters and a few volatile oils.
ISOLATION OF ACTIVE PRINCIPLES

For any drug to produce a real as opposed to an imagined response, it must at the very least interact at the molecular level with either a component of the body or an infectious micro-organism. A drug may also interact elsewhere to produce an undesirable effect. As natural products such as plants contain a complex and variable mixture of chemicals, the risk of unwanted side effects occurring is always high. The simplest way to minimise this is to isolate the component that produces the desired response. While this may be obvious today, it was not the reason behind the successful isolation of active principles in the second decade of the nineteenth century. The sole motivation for this was the need to identify adulterated plant products by determining the amount of active principles in them. Only after this could be done was it recognised that the amounts of these principles varied both quantitatively and qualitatively throughout the life cycle of the plant, being influenced by changing climatic conditions. The time of harvesting, adequacy of the drying process, and nature and duration of storage further influenced the composition of these chemicals.

The inability to identify and quantitatively analyse the active substances in herbal medicines before the nineteenth century means that there is uncertainty surrounding the nature of all herbal remedies used before this period. It may also explain why there was no effective challenge to the beliefs of the ancient healers other than when mineral drugs were recommended, which was from the tenth century onwards. Unlike herbs, these could be purified and analysed due to their ability to withstand high temperatures. They could also survive exposure to strong acid or alkali to form salts that could be refined by crystallisation.

The uncertainty about the identity and quality of ancient herbal remedies meant that they could never be properly assessed in the clinic. Added to that was the total failure of reports about their medicinal applications to even consider, let alone apply, the various factors that we now know must be taken into account when evaluating any drug in the clinic, irrespective of its origin. Thus the ancient and mediaeval medical literature that we have inherited reveals no awareness of that classic example of mind over matter, the placebo effect. Nor does it take into account how patients were selected or how the nature of their ailment was assessed prior to treatment. Indeed, that would have been fraught with difficulty for those whose diagnostic skill was restricted to assessing which of the four bodily humours was present in excess or was deficient. In addition, there was never any attempt to eliminate bias on the part of the attendant physician. As to any consideration of the dose, or frequency and duration of the treatment, little was ever written. Side effects of medication were sometimes even considered to be evidence of their efficacy and at other times a sign of their failure. As for defining what could be considered as a successful outcome, again little was ever written. All of this means that claims made for traditional remedies in the past have no validity.

It is not a matter of commenting unfairly on what was done in the past by applying our modern understanding of the subject. The reality is that out of the myriad of plants and minerals found on this planet, remarkably few possess the ability to relieve disease when rigorously evaluated by the criteria of modern, evidence-based medicine which sets high standards for the conduct of clinical trials. Few minerals are able to penetrate across biological membrane barriers within the body, and if they do they are often highly toxic. As for the chemicals in plants, their very role as irritant or toxic materials that provide a defence against foraging predators is singularly inauspicious with regard to healing the sick. Plants did not evolve for the purpose of producing medicines! There are an estimated 320 000 plant species currently growing in this planet, with perhaps as many as 25 000 of these having been utilised in various systems of folk medicine. Many active principles were extracted over the last two centuries, yet only 120 or so are currently in use around the world. For any single country, the number is much lower; in the United Kingdom fewer than 40 are in regular use in mainstream medicine.
Pharmacologically active compounds from plants became available at the beginning of the nineteenth century through the technique of solvent extraction. Access to pure alkaloids and glycosides meant that patients received exactly what the prescriber intended, rather than a product of highly variable composition, even if it was not adulterated. Furthermore, the ability of alkaloids to form water-soluble salts meant that parenteral medication became a reality in the middle of the nineteenth century when the hypodermic syringe was developed.

As more alkaloids and other pure products were isolated, it became obvious that most of these were highly toxic when administered to patients. This is hardly surprising in view of their role as poisons to protect the plants from foraging predators. While the ancient materia medica has certainly been the source of a variety of valuable pharmacologically active principles, there has been a tendency to underestimate their toxicity. Plant-derived substances have a role to play, though they tend to be among the most toxic drugs in the therapeutic armamentarium. It is noteworthy that the herbs promoted by the retailers of herbal nostrums are supplied in formulations containing low doses for precisely that reason. Homeopathy developed in the early nineteenth century by taking this approach to the extreme by diluting toxic plant products to such an extent that patients ingested no active drug at all.

The influence of plant products on drug research has been disproportionate to their therapeutic value. During the twentieth century, both academic and commercial researchers spent time in seeking out novel preparations employed by native tribes or users of folk medicines. The paucity of useful outcomes from this is hardly surprising, for the rationale of treating the sick with such products is inherently unsound since in most cases the products were chosen on account of a perceived property such as taste, smell, colour or shape. Alternatively, plants were selected because of a dramatic effect like purgation, emesis, sneezing, increased urination, salivation, sweating, stupefaction, hallucination or mood alteration. Truly effective modern drugs such as antibiotics, antivirals, statins, hormone derivatives, and the like, are often devoid of any perceptible effect that is related to their action. If research into plant products is to be more successful in the future, rigorous criteria for their selection for investigation will need to be developed.

SYNTHETIC DRUGS

The initial enthusiasm for the use of alkaloids and other active substances from plants in the first half of the nineteenth century was followed by a period of therapeutic nihilism during which many medical practitioners came to doubt the value of drug therapy. That was, however, to change with the rapid development of synthetic organic chemistry to meet the demands of the emergent synthetic dyestuffs industry in the wake of the serendipitous discovery of mauveine by William Perkin in 1856. This meant that for the first time in history it was not necessary to rely on nature to provide new drugs. This did not mean that natural products were no longer important. On the contrary, these have remained the progenitors of many drugs developed since then, but the universal success of the synthetic hypnotic chloral hydrate in 1868 and then sodium salicylate seven years later resulted in the emergence of the industrial production of synthetic drugs. At first these were discovered in universities, but by the end of the nineteenth century the leading pharmaceutical companies were developing new drugs in their own laboratories. A significant event at this time was the arrival on the scene of industrial pharmacologists and chemists specialising in drug discovery.

Initially, chemists simply made minor changes to the chemical structures of natural products by altering functional groups that had been identified. Some of these were converted to esters or ethers, while others were alkylated, hydrolysed, oxidised or reduced. By the end of the nineteenth century, the chemical structures of alkaloids were beginning to be determined. This meant that analogues could be designed in which only essential parts of the molecule were retained. In the second half of the twentieth century, the process of analogue design became
highly sophisticated as medicinal chemists wrestled with structural manipulations that both affected transportation of drugs to their intended sites of action and also ensured optimal docking on arrival there. In pursuing their manipulations chemists occasionally stumbled upon molecules that exhibited wholly unexpected activities, thereby extending the available range of pharmacophores. All this activity resulted in many hundreds of valuable new therapeutic agents that generated fortunes for their industrial exploiters. Patients, too, benefited and by the end of the twentieth century drug therapy had become the major mode of bringing succour to the sick. There still remained a strong dependence upon naturally occurring molecules as sources of inspiration for the design of new drugs, thanks to the discovery of mammalian hormones and also antibiotics.

**DRUG PROTOTYPES**

It was not until demonology, astrology and humoral imbalances had been thoroughly discredited that drug research could make progress. Yet until the late nineteenth century its scope had been limited to testing and evaluating readily available natural products. Once organic chemistry had finally developed sufficiently for a limitless range of new drugs to be synthesised, researchers began to modify the chemical structures of natural alkaloids in the hope of finding better products, or attempted to synthesise substitutes for them in order to overcome shortages and high costs of production.

Many of the successes of drug research have involved the preparation of analogues of natural products or synthetic compounds possessing some form of exploitable pharmacological or chemotherapeutic activity. It is helpful to describe the first pure compound to have been discovered in any series of chemically or developmentally related therapeutic agents as a drug prototype. In certain cases, there has been no perceived need to develop a prototype further. Such prototypes continue to serve as medicinal compounds in their own right, but others have been rendered obsolete by the analogues derived from them. In some instances, both the prototype and its analogue even compete in the market-place. In a recent study, nearly 250 drug prototypes were described, from which 1200 medicinal compounds were derived.2

Until the middle of the twentieth century, most drug prototypes were derived from plants. For a period thereafter, the major sources of these were micro-organisms that yielded antibiotics. Throughout the century, however, prototypes were being isolated from biochemical sources and this eventually became the principal route for discovering novel drug prototypes. Spurred by major advances in physiology, biochemistry, microbiology and pathology, chemists were at last provided with the full scope to apply their newly acquired skills. Hormones and antibiotics alike were then altered to limit undesired effects and to permit their administration as medicines that could survive in the human body long enough to exert a useful action. Indeed, the twentieth century was the first in which drug therapy contributed more to the progress of medicine than did any other single factor.

The start of the twentieth century had been marked by the isolation of the first pure hormone, epinephrine (adrenaline), yet analogues of it were not developed until after the introduction of the alkaloid ephedrine in 1926, when it was recognised that it was a chemical analogue of epinephrine with some advantages when used therapeutically. By that time, thyroxine, insulin and acetylcholine had also been isolated, followed by the corticosteroids and sex hormones in the 1930s. This formed the basis for some of the greatest achievements of medicinal chemists in the twentieth century when they later prepared analogues of epinephrine and cortisone in which undesirable effects on the body were largely overcome while leaving desired effects unaltered or even enhanced. This successful exploitation of hormones in drug research had much to do with their natural role, which is to regulate physiological function, not to disrupt it. With plant poisons it is the converse, since their role is to protect plants
against foraging predators. However, the hormones had presented problems when they were selected to serve as drugs. Not only did they often occur in only trace amounts, making isolation difficult, but they were also often chemically unstable. Additionally, when employed as drugs, they lacked sufficient specificity of action, and it required considerable effort by medicinal chemists to develop them into selective therapeutic agents.

During the year 2004, the 25 millionth compound was listed in the registry of the Chemical Abstracts Service. The availability of so many compounds prepared for a wide variety of purposes other than drug therapy has provided opportunities for serendipitous discoveries of unexpected pharmacological activity. In addition, the screening of ever expanding collections of synthetic compounds prepared by drug companies for earlier projects has provided another potential source of novel prototypes.

The first major break with reliance upon natural products as drug prototypes was inspired by the development of synthetic dyes. This was the introduction in 1904 by Paul Ehrlich of the antitrypanosomal agent known as Trypan Red. Developed as a result of screening over one hundred dyes in a quest to find one that would bind to and kill the trypanosomes that caused the tropical disease known as sleeping sickness, Trypan Red proved toxic to the optic nerve and was reserved for veterinary use. Five years later, Ehrlich evaluated arsphenamine, an organic arsenical drug that cured syphilis. It was an analogue of Atoxyl®, a highly toxic compound used for treating sleeping sickness and originally introduced as an anticancer drug in the unsubstantiated belief that it would be less toxic than arsenic trioxide as it was an organic compound. Further dyes and arsenicals were developed from those introduced by Ehrlich, leading to effective chemotherapy against sleeping sickness, malaria and a variety of bacterial diseases.

By the middle of the twentieth century the majority of drug prototypes were no longer gleaned from the plant kingdom. Biochemists had come to grips with delicate molecules harboured within cells and vesicles or circulating in body fluids. The animal organism had now become the single greatest provider of drug prototypes. It has retained that role, rivalled only by prototypes obtained from fungi and other microbes. In the 1940s a therapeutic revolution occurred when the first antibiotics were introduced. However, the vast majority of the antibiotics that have been discovered are, like the products from plants, too toxic for therapeutic application. This toxicity is a reflection of their role in the battle for survival of their host organisms in the natural world.

The range of drug prototypes now includes hormones, cellular metabolites and other substances found in the human body, as well as vitamins and antibiotics. Chemists have used their skills to create new drugs by synthesising analogues of those that exhibited desirable characteristics such as greater safety or efficacy, enhanced stability, improved selectivity or duration of action, superior absorption and distribution characteristics, and so forth. In the closing years of the twentieth century, attempts to generate novel prototypes from which new medicines could be developed moved in a new direction with the introduction of combinatorial chemistry. This permitted the automated synthesis of vast numbers of molecular variants that were fed into high-throughput screening systems in the hope of finding active compounds. While the variants can be analogues of compounds with known activity, the possibility of now screening compounds with no resemblance to anything found in nature may further lessen the dependence upon nature as a source of drug prototypes.

ANALOGUES OF DRUG PROTOTYPES

All drugs exhibit unwanted side effects in at least some individuals. For that reason alone, attempts will always be made to find alternatives so long as it is economically viable to do so. Another reason is the need for commercial companies to hold patents on their products. As these have only a limited lifespan, companies will protect their own interests by seeking
successor products to patent. Whatever the motivation, the simplest and most frequent way of finding an alternative to an existing drug is to synthesise chemical analogues and test these.

Analogues of existing drugs could only be made once pure compounds had been isolated. Some of the first to be made were analogues of morphine, quinine, atropine and cocaine. In these particular cases, the correct chemical structure of the prototype had not yet been determined when its analogues were synthesised. Chemists merely replaced or modified functional groups believed to be present. At first, simple derivatives were made by esterification, hydrolysis, oxidation and reduction. Analogue formation became more sophisticated once the chemical structures of these and other compounds were correctly established and when recognition of the importance of the physical properties of the drug became widespread, especially in the second half of the twentieth century.

Structural modification was used to change lipophilicity in order to control the ability of drug molecules to penetrate biological membranes by passive diffusion; e.g. alkyl side chains were lengthened or shortened to make compounds less or more polar and thus increase or decrease the likelihood of penetrating into the brain. This was followed by a growing awareness of the relevance of constraints placed on organic molecules through the degree of ionic dissociation, which could render potential drugs useless if the charged molecular species was that mainly present in the body. Careful placing of chemical substituents was found to alter radically the tendency of molecules to ionise, thereby enhancing their pharmacological activity.

Apart from altering the physicochemical properties of drugs to develop novel analogues, chemists have also manipulated the molecular shape or electronic distribution within drugs with a view to modifying molecular interactions at their site of action. Unfortunately, such change has frequently proved to be incompatible with the desired physical properties that affect the distribution and metabolism of drugs within the body. This is the principal reason why the vast number of analogues fail to behave in a satisfactory manner.

It is exceedingly difficult to develop new drugs that are both safe and effective. It may take years of intensive research before structural alterations to a prototype or an existing drug will provide a compound that can satisfy the stringent safety requirements universally applied to all new pharmaceutical products. Even when that much is achieved, sometimes all that is obtained is a novel compound that is no more than a commercial success. Such products have been much maligned for their failure to confer any therapeutic advantage over their predecessors. Nonetheless, there have been several instances in which drugs that appeared to be unnecessary variants of an existing product have been found from long-term studies to be safer, more efficacious or sufficiently different in their mode of action as to constitute important therapeutic developments. This factor alone could justify the marketing of almost any variant that is at least as safe and efficacious as the existing product, but the cost of bringing a new drug to the market is now so high that companies are reluctant to invest in any drug that does not have some significant advantage over its rivals. In the past, this restraint was not so powerful and consequently there are still redundant drugs on the market.

REFERENCES
In 1975, Ralph Solecki of Columbia University described excavations at the Shanidar Cave in the Zagros Mountains of northern Iraq, where he had unearthed 60 thousand year old Neanderthal bones laid out in the foetal position. Alongside were clusters of pollen grains from 28 species of flowers. As seven of these were recognised to be medicinal herbs, it was suggested that this could explain their presence. Solecki dismissed the possibility that the Persian jird, *Meriones persicus*, a rodent that stores seeds and flowers, may have contaminated the site by its burrowing. A later investigation by Jeffrey Sommer of the University of Michigan revealed that the types of pollen grains in the Shanidar grave were also found in jird burrows. While this does not prove contamination had occurred, it does mean that further investigation is required before firm conclusions about the Shanidar Cave can be drawn. Moreover, the presence of a few plants that are reputed to possess medicinal properties does not in itself mean they were used for such purposes. They could simply have been food, especially as ancient medicines often originated as components of the diet that were deemed to have beneficial effects on health.

There is indirect evidence of drug use in the Neolithic period, which began around 11 thousand years ago. Among remains of plants found in the Spirit Cave in the north-west of Thailand were seeds of the mildly psychoactive betel nut (*Areca catechu*), placed there between 7000 and 5500 BC. The earliest direct evidence for human consumption of betel nut comes from the Duyong Cave in the Philippines, where a skeleton from 2680 BC was found buried beside shells containing lime. This is reminiscent of the practice still seen in India, of wrapping the nut in betel leaf (*Piper betel*), adding lime (thus liberating the readily absorbable free base of arecoline), then chewing. As with modern users of betel nut, the teeth of the Duyong Cave skeleton were blackened.

Warming of the Fertile Crescent in the Middle East after the end of the last Ice Age permitted agricultural development. A transition from hunting and gathering to settling in villages developed over a six thousand year period, spreading to Europe, the Indus Valley and onwards, with parallel developments occurring in Central America and Northern China. Following upon the domestication of the horse by the fourth millennium BC, our ancestors acquired the ability to travel great distances with alacrity, a freedom the slow-moving ox had denied them. The consequences of this were profound. Trade developed, cities were established and conquest ensued, all of which created a need for law and order that in turn stimulated the development of writing in Mesopotamia and Egypt. With improved communications and the interchange of ideas, knowledge of herbs that could be used as spices, to treat disease or to offer some other beneficial action was no longer confined to isolated communities, and trading began in those products that could be preserved.

ROCK ART

Even before the appearance of writing there was another form of communication that has preserved information about the lifestyle of our prehistoric ancestors. This was rock art,
consisting of paintings and inscriptions on rocks either exposed to the elements or protected inside caves. In 1958, the American anthropologist Thomas Campbell drew attention to intriguing parallels between aspects of the ancient American Indian cult of the mescal bean (*Sophora secundiflora*) and drawings on the walls of caves near the confluence of the Pecos River and the Rio Grande in the south-west of Texas.6 The drawings depicted dancers with bows and arrows, hunting and deer. Campbell also noted that mescal beans had repeatedly been found in nearby caves over the preceding 25 years and in one case had been radiocarbon-dated as originating in the period 7500 BC to AD 200. In another cave were remains of peyote, a mescaline-containing cactus (*Lophophora williamsii*), and Mexican buckeye (*Ungnadia speciosa*), both known to be psychoactive.7,8 The Pecos River cave drawings are now believed to be only four thousand years old, but that has not deterred the publication of reports5 about prehistoric cave drawings involving psychoactive drugs since the appearance of the paper by Campbell.6 There are many who dismiss such claims as being highly speculative and it should be pointed out that cave art with similar content to that of the Pecos River drawings has been discovered in parts of the world where there have been no findings of mescal beans or other psychoactive materials. Just as controversial are reports of cave paintings that are said to depict shamanic healing rituals in which the medicine man entered a trance-like state that may have been induced by either hyperventilation, ritual music and dancing, or drugs.9 It has, for example, been proposed that central Saharan Desert cave paintings from the period 7000–5000 BC represent the ritual use of hallucinogenic mushrooms since they portray scenes of harvesting, adoration, dancing and the offering of mushrooms.10

**DRUGS THAT ACT ON THE MIND**

The historical record of drugs consumed by our Neolithic ancestors could have been unduly influenced by those that exhibited an effect on the mind, such as mescal bean, peyotl, betel, the poppy, alcohol, coca, cannabis, tobacco, soma and pituri.11,12 The social and recreational use of these drugs is associated with artefacts, in much the same way as nicotine and alcohol are today involved with pipes and liquor glasses respectively. Drugs employed for medicinal purposes, by contrast, do not leave enduring evidence of their consumption, particularly if they were also considered to be foods rather than medicines – as is still the case today among North American Indians who cultivate certain plants for consumption as food, yet also employ them for therapeutic purposes.13 It is hardly surprising therefore that archaeologists have found more evidence of drugs used for social and recreational purposes than those selected as medicinal substances. It is noteworthy that when written records appeared in the third millennium BC onwards, psychoactive drugs did not appear to be dominant.

Not only has it been suggested that the original use of drugs was in religious rituals14 but it has even been claimed that early religious practices arose from the cultic use of psychoactive drugs.15 This may be controversial, but the role of wine in religious practice in ancient times is beyond dispute if we consider the Greek Dionysian festivities, the Roman Bacchanalia and the importance of wine in Judaism and Christianity.

**Alcohol**

The most widely consumed drug is, of course, alcohol. It is not surprising that alcoholic beverages were among the earliest of drugs to have been discovered since their effects must have been readily perceived within a short period of administration. This remained a basic necessity for drug discovery until the isolation and testing of active ingredients from plants in the nineteenth century. When toxic effects also occurred with little delay, the drug was considered to be a poison. However, if these were not manifest until a considerable time had
elapsed, there was little likelihood they would be connected with the drug. This remains a problem for those who consume herbal remedies, but it also applies to modern synthetic drugs. In recent years, thousands of people were prescribed drugs such as thalidomide, benoxaprofen, practolol and others without anyone being aware of the damage these caused because of the delay before it happened. It was left to astute physicians to detect the link between these drugs and their toxic effects.

Alcohol appears to have first been consumed in the form of wine. Evidence of wine being available over seven thousand years ago has been obtained from chemical analysis of yellow residues found in six vessels sunk in the floor of a mud brick building of the Neolithic Hajji Firuz Tepe village unearthed in the Zagros Mountains of western Iran. The capacity of each vessel was nine litres, indicating that organised production had taken place. Infrared, high-performance liquid chromatographic and chemical analysis of the yellow residue indicated that it consisted largely of calcium tartrate together with resin from the terebinth tree. The calcium tartrate could only have come from grape juice as there is no other common natural source. The juice would have fermented into wine on storage, while the resin served as a preservative to prevent despoliation by bacteria that convert wine to vinegar.

Excavations further south in the Zagros Mountains uncovered a military centre known as Godin Tepe, where earthenware jars from 3500 to 3000 BC were found. Again, deposits of calcium tartrate were present. By this time wine and beer were being prepared throughout the Aegean.

It is not clear whether the production of beer preceded that of wine. A 4000 BC seal depicting two people drinking beer with a straw (characteristically used to filter out the mash floating in the beer) was discovered in the 1930s during excavations of the Tepe Gawra site near the ancient city of Nineveh in north-east Iraq, and beer is frequently featured in Sumerian drawings and text of the third millennium BC.

**Opium**

Alcohol may have been the most frequently consumed drug in history, but the most effective medicinal drug available before the dawn of the twentieth century was opium obtained from the poppy, *Papaver somniferum* L. – one of the oldest cultivated species known. Claims that opium was used in the Neolithic period should be viewed with extreme caution. They are based on the finding of seeds of the closely related *P. somniferum* subsp. *setigerum* in Germany at the Danubian settlements, the location of the first central European farms around 4400–4000 BC. Much greater quantities of poppy seeds have been found in northern France and farming settlements on the shores of lakes in Switzerland and surrounding areas, which date back to 3700–3625 BC. Both *P. somniferum* and its subspecies *setigerum* produce morphine and related narcotic compounds, though the former generates greater amounts. However, the seeds of both plants contain insufficient amounts of active alkaloids to produce any narcotic effect. The greatest concentration of active alkaloids is to be found in the latex exuded from the unripe capsule, which is dried to prepare opium. The seeds from the Neolithic period were probably used for the production of oil, as they certainly were in later times.

Evidence of what appeared to be early opium use came from the Cueva de los Murciélagos (Cave of the Bats), situated 50 metres above a ravine near Granada in Spain. In 1868 Góngora y Martínez reported that he had found poppy capsules in esparto grass bags in this ancient burial site. The capsules were radiocarbon-dated in 1975 as having originated about 4200 BC, but this was later revised to around 2500 BC. It thus appears that there is no reliable evidence for the use of opium prior to the appearance of writing.
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Pre-Hellenic Civilisations

Writing developed in Mesopotamia and Egypt six thousand years ago in the form of crude pictographs representing everyday objects, but gradually these were modified to represent ideas and ultimately sounds. Considerable progress was made in the nineteenth century in transcribing the wedge-shaped cuneiform text introduced by the Sumerians around 3100 BC, as well as the hieroglyphics of the Egyptians. Consequently, there now exists substantial knowledge of the lifestyle of these civilisations, including how they used and administered drugs. However, progress in identifying individual drugs has been more difficult.

A major source of information about drugs used in Mesopotamia is provided by the 660 tablets of clay recovered in the 1920s from the library of the palace in Nineveh of the last king of Assyria, Asshurbanipal (669–626 BC). Most of them appear to have been written around 1700 BC. They were among 20 thousand tablets unwittingly preserved by being baked when invaders set the palace on fire. The tablets were deciphered by R. Campbell Thompson, who established that many were copies of much older texts, with some of the drugs possessing Sumerian names from the third millennium BC.¹

Thompson painstakingly sought to identify each plant mentioned in the tablets, both from the general context in which its name appeared and by reference to other cuneiform texts. He then adduced philological evidence from other languages before comparing his conclusions with the findings of other scholars, taking fully into account the ancient, mediaeval and modern usage of drugs in the Middle East. His work remains important, despite valid criticism that there were many errors in his identification of the 115 or so drugs.² A number of his findings were revised in a posthumous publication,³ but his mistaken belief that the wild poppy, *Papaver rhoeas* L., produced narcotic substances persisted and has continued to mislead those who are unaware that morphine is not formed in this species. This has resulted in the widespread belief that opium was used 5000 years ago in Sumer. The situation has been further confounded by the suggestion that a third millennium BC ideogram on a clay tablet from the holy city of Nippur depicted *HUL GIL*, the plant of joy, this allegedly being the opium poppy. That opinion was originally expressed in a personal communication from a professor at Yale.⁴ There is no evidence to support it.⁵

The herbs cited in his posthumous publication that Campbell Thompson claimed to have identified include aloes, ammi (toothpick plant), anemone, anise, asafoetida, balm of Gilead, beetroot, black cumin, black hellebore, bitter or black nightshade, cannabis, carob, cassia, castor oil, cedar, chamomile, chasteberry, citron, colcynth, cornflower, cress, cumin, darnel ryegrass, date palm, elder, fennel, fig, frankincense, galbanum, garlic, ginger, heliotrope, henbane, hound’s tongue, laurel, leek, liquorice, mandrake, meadow saffron, mint, mustard, myrrh, myrtle, nettle, oak, pellitory, pine, pomegranate, poppy, rocket, rosemary, Syrian rue, saffron, squirting cucumber, styx, sweet flag, sycamore fig, tamarisk, thistle, thorns, thyme, turmeric, white hellebore, winter cherry, willow and wormwood. Caution must be exercised in accepting his conclusions, though many of these plants are used to this day in herbal medicine – albeit with little more evidence of their efficacy than was available to the inhabitants of Mesopotamia. It is interesting to note that the medical texts distinguished between different

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parts of the plant. This indicates an early awareness that this was an important aspect of using herbal medicines.

Many of the plants mentioned by Thompson had multiple uses – a feature of all ancient systems of medicine since they held that all ailments were different manifestations of the state of disease. Thus any remedy that was believed to expel the demons from the body or ameliorate an imbalance of humours would inevitably have a broad spectrum of application. This did not change until the emergence of pathology as an independent medical science. As for the actual uses mentioned by Thompson, they are diverse and include treatment of a wide range of gastrointestinal, urinogenital, pulmonary and abdominal problems, as well as external afflictions such as scorpion stings, bruising and blistering, irritation of the eyes, ears, mouth, feet and so forth.

In addition to the cuneiform tablets from Nineveh, there are many others, including 420 translated by Franz Köcher. From these sources we learn that there were two types of healers, namely sorcerers who belonged to the clergy, and physicians. The former, known as the ashipu, believed that disease was caused by demons and employed magic, incantations, charms and exorcism to expel them, while the latter, the asu, followed a therapeutic tradition of using animal, vegetable and mineral remedies in a pragmatic manner. There was often interaction between these groups, but it is clear that an independent therapeutic tradition had developed in Ancient Mesopotamia. This was an old tradition that originated in the prehistoric world and it appears likely that it passed to the Egyptians.

There may be problems with the identity of the drugs cited in ancient tablets, yet they can provide valuable information about how drugs were prepared and administered. Samuel Kramer wrote about a four thousand year old clay tablet excavated at Nippur that may be the oldest pharmaceutical text known. He and chemist Martin Levey translated the tablet and found it contained more than a dozen prescriptions with ingredients from animal, vegetable and mineral sources, but there was no indication of their uses. Of the minerals, both salt (sodium chloride) and saltpetre (potassium nitrate) were preferred, while from the animal kingdom milk, snakeskin and tortoise shell were favoured. However, most of the medicaments were of vegetable origin, a situation that persisted until modern times. Amongst these were said to be cassia, myrtle, asafeotida and thyme. There was no mention of the quantities to be incorporated, but all parts and secretions of the plants were used for making the prescriptions. In some cases, several plants were treated with wine and then mixed with cedar or similar oil to prepare soothing salves. The liquid formulations were obtained by decoction of plants in boiled water, followed by addition of alkali or salts that may have enhanced the extraction process. For prescriptions administered by mouth, the pulverized ingredients were taken up in beer. Sometimes the ingredients were specially treated, such as the burning of soda-rich plants to free the alkali ash from vegetable matter. There was even a degree of chemical interaction, as in two prescriptions where fats were combined with alkali to form a soap. The cleansing and antibacterial action of this when applied directly to infected wounds was probably of more benefit than the odoriferous ingredients of other prescriptions in the clay tablet.

EGYPT

The most useful record about drugs used in Ancient Egypt is a document purchased in Thebes in 1872 by the Egyptologist Georg Ebers. Generally known as the Ebers Papyrus and dating back to around 1550 BC, this lists more than 800 prescriptions then in vogue, including most of the drugs cited in Thompson’s interpretation of the medical tablets recovered from Nineveh. This would be due to the trade routes between Egypt and Mesopotamia, resulting in cultural exchange. Both sources include elements dating back to the third or fourth millennium BC. In the Ebers Papyrus, there is once again a problem with the identification of specific herbs, but there are many prescriptions incorporating culinary materials such as dates,
figs, grapes, pomegranates, roasted barley, wheat, honey, sweet beer, wine and milk. Spices abound, including absinth, cassia, caraway, coriander, cumin, fennel, gentian, juniper, peppermint and thyme. Fats are also present, albeit from unusual sources such as cats, crocodiles, geese, hippopotamuses, lions, oxen and serpents. Among the oils are cedar wood, linseed, turpentine and other essential oils. There are also locally obtained minerals such as common salt, natron, alum, red and yellow ochres (iron oxides), as well as salts of lead, copper, mercury and antimony. Many of the prescriptions are for skin and eye complaints, as in the tablets from Nineveh, while remedies for gastrointestinal complaints are also common. Once again, the former doubtlessly reflects the hot, sandy environment and presumably the latter is related to the unwholesome diet of those days.

The prescribing of soothing balms for skin problems and purgatives for what appears to be constipation indicates that there was a strong empirical element in both the Mesopotamian and Egyptian systems of medicine, while the preponderance of spices and culinary herbs supports the view that drugs were initially considered to be foods that had some beneficial value. However, what lay behind the selection of the herbs?

Many plants contain substances that will irritate one part or another of the gastrointestinal canal, from the mouth to the rectum. The response to these substances, and consequently their application, will depend on which region is irritated, varying from salivation, or emesis, to purgation. If the elements of magic, superstition or religion are involved rather than pragmatism, the scope for innovation is immense. It does not, however, augur well for efficacy, and therein lies the problem for those who look to herbal remedies as a source of new drugs. It is conceivable that some might be, but much effort will be wasted examining worthless nostrums in the vague hope that something of value will be found.

Whereas in Mesopotamia the treatment of the sick was in the hands of two distinct classes of healers, the priestly ashipu and the therapeutic asu, most physicians in Egypt were priests. Imhotep, the earliest physician known to historians, lived during the Third Dynasty of the Old Kingdom around 2650 BC and was High Priest of Heliopolis. At that time, elements of religion and magic were closely intertwined with drug use, incantations routinely being uttered prior to administration in order to confer the healing property upon it. Without such a spell being cast, the drugs would in most cases have been ineffective. Modern knowledge of the placebo effect, whereby psychological elements influence the response to therapy, suggests that this greatly contributed to any success of the Egyptian priest–physicians. Although there were some herbs that did have real properties, such as the ability of castor oil to produce purgation, the likelihood of recognising such effective remedies would have been diminished as the outcome of their administration was difficult to distinguish from the many worthless herbs that were introduced.

Medicine acquired such a high status in Egypt that its physicians were sought out by leaders of surrounding nations. There was considerable specialisation in the treatment of different parts of the body, further increasing the demand for physicians. Although there were some who were not priests, they lacked the influence of the priest–physicians. All, nevertheless, were ever ready to administer a wide range of herbs. Regrettably, it was not the tradition of the asu of Mesopotamia that was passed to all subsequent generations by the Ancient Egyptian priest–physicians, but rather a heritage of mainly worthless remedies. Most of these are still being sold in our modern shopping malls as ‘herbal’ or ‘natural’ medicines.

Finally, it should be noted that claims that traces of morphine had been found in the tomb of the Egyptian chief royal architect Kha, who died in 1405 BC, have been disproved by modern chemical analysis.

INDIA

Recent scholarship has established that not only were there trade links between Mesopotamia and Egypt at the beginning of the second millennium BC but that the Silk Road to China was
also being established. Due to the immense distances involved, no single individual was able to travel from the West to the Far East. Although goods were exchanged, the transfer of knowledge and culture was therefore erratic and unreliable. Consequently, there are both similarities and differences in the ancient medical systems of the East and the West.

There are many who believe that the four Vedas that constitute the Brahmanic basis of Hinduism could have been compiled by the second millennium BC and that some of the mantras in them trace their origins back a further one or two thousand years. A recent challenge to established concepts of Indian history could further complicate the dating of the compilation of the Vedas.

Each of the Vedas covers the role of ritual in a different sphere of life. The last, the Atharvaveda, is held by believers to have originally contained the basis of Ayurvedic medicine, which was rearranged and revised perhaps around 800 BC in samhitas (treatises) by the physician Charaka and the surgeon Sushruta. Charms for expelling demons are to be found in their works and there is a god of medicine, Dhanvantari. The Charaka Samhita may not have been written in its present form until the first century AD. It refers to over 300 vegetable drugs, the remainder being from animal and mineral sources. The current version of the Sushruta Samhita might date to the seventh century AD. Although a text on surgery, it mentioned nearly 400 vegetable drugs. The number of plant medicines cited in these two works is greater than that of any other ancient people who left a record of such matters. However, it does not rest there, for in Sutra 26 the Charaka Samhita asserts that there is nothing in the world that cannot be used as a medicine, so long as an appropriate use can be found for it!

Ayurveda considers man to be born in a state of humoral balance that is gradually lost through changes in diet, lifestyle and environment. The humours are wind, bile and phlegm. In some traditions there is a fourth humour, blood. To restore a healthy balance in those who are sick requires not merely the use of appropriate drugs but also dietary change, controlled breathing and spiritual meditation. There is an obvious similarity to the Greek system of humoral medicine insofar as both are holistic in aiming to restore a healthy balance of humours. In both there is liberal use of emetics, purgatives, enemas, sneezing powders and the like, not to expel demons but rather to eliminate excess of undesired humours. This would hardly be surprising if the writings upon which Ayurveda is based were compiled hundreds of years after the Hippocratic Corpus of the fifth century BC, but if the concept of the humours originated before 2000 BC, a major revision of medical history would be necessary. The matter has not yet been settled, but from the perspective of the history of drug discovery, the debate is of limited concern since humoral medicine was nothing other than an impediment to the discovery of efficacious drugs.

As with several other ancient medical systems, that of India has been associated with the use of a psychoactive drug. In this instance it is soma, a substance featured in the oldest of the Vedas, the Rig Veda. Many suggestions as to its identity have been made, including the celebrated claim by Gordon Wasson that it was a psychedelic mushroom, Amanita muscaria. More recently, it has been suggested that soma consisted of a mixture of herbs including Syrian rye, Peganum harmala, the substance originally proposed to be soma in 1794. This new claim, however, depends on the assertion that soma was introduced into Northern India by Aryan invaders. However, the proponents of the antiquity of Vedic medicine strongly deny such an invasion ever occurred. From this, the reader may conclude that unravelling the history of early drug use in India is no simple matter. A thorough investigation of the subject is required.

CHINA

During the Han Dynasty in China (206 BC–AD 220) there was a proclivity to fabricate history in order to elevate the reputation of the state. Mythical figures were said to have rescued the
world from chaos, such as the inventors of fire, writing and agriculture. The latter was supposed to be a peasant who became the Emperor Shen Nung in the third millennium BC and it was claimed that valuable information about the use of drugs could be gleaned by scouring the pages of his herbal, *Pen Ts’ao Ching* (*Classic of Materia Medica*), in which he described how he tested more than 300 herbs upon himself. Acceptance of this story has persisted in China where Shen Nung is revered as the Father of Medicine, but in fact this herbal was written during the last years of the Han Dynasty and then modified in the sixth and seventh centuries. By this time, it would have been influenced through contacts with India and the Hellenic world and so diminished in value as a guide to the early use of drugs.

The concept attributed to Shen Nung that herbs have properties of inducing warmth or coolness will strike a chord with anyone familiar with the writings of Aristotle (384–322 BC). In the absence of reliable evidence to the contrary, the concept must be considered as coming from Aristotle. Another text that is just as prominent in Chinese medicine is the *Huang Di Neijing* (*The Yellow Emperor’s Classic of Internal Medicine*), written around the same time as *Pen Ts’ao Ching* but supposedly authored by a fifth millennium BC ruler. 18 This introduced the concepts of yin and yanz, the forces that must be balanced for the maintenance of good health. Yin is a negative or passive force, while yanz is positive or active.

The best known of the Chinese herbals is *Pen Ts’ao Kang Mu*, which was compiled during the Ming Dynasty by Li Shih-Chen (1518–1593). Featuring nearly 2000 herbs, as well as minerals and animal products, it summarised all that was known about Chinese medicine at that time. Since then, the number of herbs used has increased to around 5000.

**Cannabis**

Although native to Asia, it is generally accepted that *Cannabis sativa* L. was first cultivated in China around 4500 BC when its seeds served as a grain crop. Soon after, its decaying stems became the source of hemp fibre for making clothes, ropes and fishing nets. However, reports of early hemp use in China are open to question either because of inadequate identification of fibre remains or because they relate to impressions on pottery and other objects. These are assumed to have been made by hemp since other fibres, except the much finer silk, are not found in the region. Such cord impressions have been seen on material from 4000 to 3000 BC unearthed in Taiwan and also on similarly dated pots found in Henan Province, eastern China, at an archaeological site of the Yang-Shao culture, the first civilisation to manufacture pottery. Outside China, clay shards showing impressions of what are thought to be *Cannabis* seeds from around 3000 BC were discovered during excavations at a Linearbandkeramik Culture site north of the Black Sea. There are also reports of hemp fibres from objects recovered at Adouste in the south of France (2000 BC), Gordium in Turkey (800 BC) and St Andrews in Scotland (800 BC). 19 While these and many other findings indicate that hemp fibre was of importance as long ago as the third millennium BC, they do not constitute reliable evidence that *Cannabis* was being used as a drug. The frequently repeated assertion that it was then being used in China as a medicine or a euphoriant is based on the mistaken belief that *Pen Ts’ao Ching* was written in the third millennium BC by Shen Nung.

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Greece and Rome

There is a reference in the *Odyssey* to nepenthe, a drug that Helen of Troy cast into wine to induce forgetfulness in her guests who were grieving the long disappearance of Odysseus. Earlier in the *Odyssey*, Homer tells us that Helen had learned of this and other drugs during a visit to the land of the Pharaohs. The identity of nepenthe, however, is not revealed. Despite the absence of reliable evidence for the use of either opium or cannabis in Egypt before the *Odyssey* was written in the eighth century BC, both have frequently been suggested as candidates. An alternative proposal is one of the Solanaceae, such as henbane, mandrake or belladonna, all of which contain the centrally depressant alkaloids hyoscine and atropine. However, there are those who maintain that nepenthe was merely a fictional creation by Homer or an allegorical allusion to the charm of Helen.

What is particularly relevant in the present context is the allusion to Helen of Troy learning about nepenthe and other drugs in Egypt. The Greeks revered Imhotep, who was deified by the Egyptians in the sixth century BC, some two thousand years after his death, and they saw him as fulfilling the role of their own god of healing, Asklepios. Temples where the sick could receive succour were dedicated to Asklepios, just as they had been to Imhotep. The Greeks believed Asklepios was the son of the god Apollo and a mortal mother, Coronis. He was said to have learned about healing herbs from the wise centaur Chiron who dwelt at the foot of Mount Pelion in Thessaly. Asklepios then healed the sick and dying, but when he brought a corpse back to life, he incurred the wrath of Pluto who complained to Zeus that Hades was being depopulated. Zeus then struck down Asklepios with a thunderbolt. All was forgiven, however, when Asklepios was elevated to the status of an Olympian god. Whether or not he was originally a healer whose achievements led to the granting of divine status will never be known with certainty, but in the fourth book of Homer’s *Iliad*, Asklepios is presented as a prince of Thessaly in northern Greece and a healer whose sons, Machaon and Podaleiris, can cure those wounded in battle. It is noteworthy that the first temple dedicated to Asklepios was built in Thessaly. Over 300 more were constructed in Greece and elsewhere in the Hellenic world. When the sick were brought to them, their treatment consisted of fasting, sleep, hygiene and diet, supplemented by recourse to divination by the oracles. The most illustrious of the temples eventually became centres for the training of physicians. It was from one such temple in the island of Cos that physicians who wrote under the collective name of Hippocrates ensured that the therapeutic tradition of the Asklepiadae would never be forgotten.

**THE FOUR HUMOURS**

The forerunners of the apothecary in Ancient Greece were the rhizotomoi (root cutters) who recited traditional spells and followed ancient rituals as they plucked plants from the earth, such as tethering a dog to the mandrake to wrench it from the ground. They believed the beast would die as the bifurcate root uttered a shriek on being pulled out. Better a dog should die than a human. The cynic might see this as a cautionary tale intended to scare off the
unqualified herb collector, but behind their ritual was the conception that the mandrake root had the appearance of a man and hence could be used to treat infertility.

**Empedocles**

The philosopher and statesman Empedocles who lived in Aegagora in the island of Sicily around the period 460 BC was much travelled and was familiar with the lore of the rhizotomoi. This enabled him to combine the Greek mysteries and magic with medical science and natural philosophy. His doctrine of the four roots had far-reaching consequences. He argued that the universe was balanced between love and strife, the former influencing the development of superior species from primordial monsters by interacting with the four roots from which all matter is derived. Different objects consisted of different proportions of these roots; they have frequently been named ‘elements’, but Empedocles did not use that term. He identified them as earth, air, fire and water, believing they had a mystical as well as a material dimension, including magicspiritual associations with the gods. One interpretation of his writings suggests that he associated ‘earth’ with Hera (the goddess of marriage and birth), ‘air’ with Zeus (the god of gods and men), ‘fire’ with Hades (god of the underworld) and ‘water’ with Persephone (goddess of the underworld). There was also a connection with the macrocosm: ‘earth’ linked to the land, ‘air’ to the heavens, ‘fire’ to the sun and ‘water’ to the sea. Since his views applied to man as well as the natural world, they inevitably led to the idea of the four humours, but these are not mentioned in the extant writings of Empedocles.

**Hippocratic Corpus**

Hippocrates is one of the most famous figures in the history of medicine, often being called the ‘Father of Medicine’. He is said to have been born in Cos around 460 or 450 BC and was referred to by Plato and Aristotle, his younger contemporaries. About 60 medical treatises have been attributed to him, but they reveal the hand of different authors with conflicting opinions, writing over an extended period of time, mainly during the period 420–370 BC. Collectively known as the *Hippocratic Corpus*, these prose works in the Ionian dialect of Athens may have originally been housed in Cos, but they were gathered together in the great library of Alexandria around 280 BC. Even at that time there was uncertainty over their authorship.

The *Hippocratic Corpus* was compiled at the height of the classical period when philosophers were attempting to explain the nature of the world in rational terms that excluded magic and superstition. The views of Empedocles clearly had a strong influence, with the first known account of the four humours, blood, phlegm, yellow bile and black bile, appearing in the *Hippocratic Corpus*. The origins of these may be traceable to the more distant past, but they are manifestly the application of the doctrine of the four roots to the human body. As such, it was to have a negative influence on the development of physiology for over two millennia until Rudolf Virchow proposed the cellular theory of tissue organisation in 1858.

Individual contributors to the *Hippocratic Corpus* frequently contradicted each other, with one even being scathing about the views of Empedocles, but there was a general acceptance of the prevailing view that the body was in a state of balance that was disrupted in disease. Phlegm and yellow bile were considered as prime factors involved in illness, the former being associated with water and the latter with fire. Their presence could be detected when they flowed out of the sick patient. Blood was considered essential for life, although it was also viewed by some of the writers as a cause of disease when present in excess. In stark contrast to blood stood black bile, a vaguely defined malevolent humour of indeterminate composition but related to yellow bile. To complicate matters further, each humour was linked to one of
the seasons and to a phase of the life cycle of man. Despite differences in opinion as to their exact nature, the requirement for a healthy balance of humours to return the sick patient to good health provided the physician with a rational approach to therapy. Magical and superstitious factors were at last set aside.

In his writings Plato twice refers to Hippocrates as an Asclepiad. This is not true of those writers of the Corpus who travelled extensively around the Greek islands, but it is reflected in the approaches taken by the others who put stress upon first observing the patient with great care in order to determine the nature of the disease and thereby determine its course and means of treatment. There was a firmly held conviction that the body would heal itself if left alone, so treatment was often based on a change of diet as it was believed the imbalance of humours could be related to unhealthy eating and lifestyle, the latter being inherent in the Greek word *dieta*. In those times the quality of food left much to be desired and nothing was known of vitamins, so this belief in a change of diet to prevent or cure disease had some justification. Only when this approach had failed was recourse made to drug therapy. This involved either prescribing mildly acting herbs or else referral of the patient to the rhizotomoi. As it was believed that disease could be caused by one of the humours blocking an internal vessel, drug treatment often involved the administration of a herb that would clear the blockage. Cholagogues, diuretics, emetics, emmenagogues, expectorants, purgatives, sudorifics and a variety of other herbs that irritated tissues to produce muscular contraction had previously been relied upon to drive demons out of the body, so there was no shortage of herbs that could clear the various types of blockage. Remedies once thought to expel demons were now believed to expel humours. However, the emphasis now was on treating the patient rather than the disease. This was an advance on religious, magical or superstitious approaches, yet this holistic method was fundamentally flawed because it dissuaded physicians from investigating the real causes of disease and ultimately finding effective treatments. This was coupled with the typical Greek failure to undertake experimental investigation.

Aristotle

The allure of humoral medicine seems to have been irresistible. Even Aristotle (384–322 BC) was ensnared by it. His wide knowledge of plants and animals led him to recognise the power of the seed to grow into a fully developed plant. He ultimately came to believe that all objects had within them the power to change in a predetermined manner and he wrestled with the nature of that internal power. His conclusion was that the four elements were associated with four powers, or qualities, existing in two opposing sets, such as hot and cold and moist and dry. Thus he held that each of the humours consisted of one of the elements of Empedocles possessing two of the four qualities. Furthermore, these qualities were said to possess spiritual dimensions. The implications of this were far-reaching. Alcmaeon, a contemporary of Empedocles, had already asserted that disease was due to an imbalance, possibly of heat or cold brought about by an inappropriate diet. Aristotle therefore concluded that as drugs possessed elements with sets of qualities, imbalance of the humours could be treated with appropriate medication to restore any deficiencies. So far as drug discovery was concerned, the idea that the qualities of an object were a reflection of its elemental content might seem attractive in the twenty-first century, but all it did in an era when people believed there were only four elements was to muddy the waters.

THE GREEK HERBAL TRADITION

Aristotle had been head of the Academy of Athens, a forerunner of the universities, which Plato had established in 387 BC. Aristotle was succeeded by Theophrastus (c. 370–287 BC), his friend and former student. Theophrastus extended the academy and purchased adjacent
ground where he created a large garden in which his pupils could study plants, including those sent by the army of Alexander the Great from the lands conquered in the East, as far away as the River Indus in India. The knowledge thus acquired enabled Theophrastus to write two Greek texts, *Enquiry into Plants* (*Historia Plantarum*), in which he classified and described over 500 plants, and *Growth of Plants* (*De Causis Plantarum*), in which he explored the physiology of plants. These were the first publications of their type and ensured that the concepts about the four qualities developed by Aristotle would never be forgotten. They also perpetuated the myths created by the rhizotomoi that have already been mentioned, including the warning to collect peony only at night lest a woodpecker attacks the digger’s eyes.

Alexandria on the Mediterranean coast of Egypt gradually became the centre of Greek culture and the greatest minds were attracted to the city, such as Herophilus who came there from Chalcedon, a town near Byzantium, and established a medical school around 300 BC. As Alexandria was also a major trading centre, knowledge of the herbs in vogue there spread throughout the ancient world, including the emerging city of Rome, where there was an interest in Greek art, literature, philosophy and science. When Rome grew in might and wealth, the best Greek physicians went there to practise.

King Mithridates VI of Pontus (120–63 BC) on the southern coast of the Black Sea was a ruler with many enemies, having incurred the wrath of the Romans by conquering surrounding territories. Not unwisely, he feared being poisoned and sought out an antidote that could offer protection. He turned for assistance to the rhizotomist Crateuas, author of several books including the first illustrated herbal, *Rhizotomikon*. Crateuas then became personal physician to Mithridates and set about compounding a large number of ingredients, possibly over 40, into an eponymous antidote known as mithridatium. According to Galen, this was tested on slaves who had been deliberately poisoned. After Mithridates was defeated by Pompey, the formula came into the hands of the Romans. Nero’s physician Andromachus added further ingredients, as did Galen, who brought the total up to 77 and renamed it ‘Theriac of Andromachus’. The large number of ingredients, according to Galen’s thinking, ensured that the body would select one that was a suitable antidote to the poison. He even incorporated the flesh of a viper in the belief that this would offer protection against snakebites.

**Dioscorides**

Crateauas is believed to have recommended colchicum corm (autumn crocus) as a cure for gout, but it became unpopular because of its severe toxicity. When pure colchicine was isolated in the nineteenth century it indeed proved effective for gout, especially as it was now free from contamination with the veratrine that had caused much of the toxicity. Colchicine is the sole legacy of Crateuas since none of his books have survived. However, his *Rhizotomikon* may well have formed the basis of some of the text and illustrations of plants later attributed to Dioscorides in the oldest extant copy of his herbal, the sixth century illuminated manuscript in the Vienna State Library known as *Juliana Anicia Codex*. This is a copy of *De Materia Medica*, written by Dioscorides in Greek between AD 60 and 78. This herbal is the major source of information about medicinal herbs used in the Hellenic world, citing over 600 plants that remained the mainstay of herbal medicine through the ages. Dioscorides provided a description of each herb and its habitat, discussing its humoral qualities and its characteristics so that adulteration could be recognised. He took great care to include not only as much information as he could about the medicinal uses of the herbs in man and animals but also reported the side effects. He also warned that the qualities of drugs were not obvious from mere inspection or tasting; they had to be tested. The approach taken by Dioscorides was essentially empirical and he had little time for theoretical speculation, but he did organise his
herbal according to the qualitative properties of the drugs, such as warming or cooling. As this made it difficult to comprehend, others reorganised the text for greater clarity after his death. Dioscorides was an astute observer and claimed to have tested many herbs on himself. It is possible that he was a physician who had travelled widely, as he claimed, for he described drugs from many lands, among which were almond oil, belladonna (deadly nightshade), bramble, caraway, cassia, cherry syrup, cinnamon, cumin, dill, fennel, ginger, henbane, lavender, liquorice, marjoram, mandrake, mint, mustard, olive oil, opium, parsley, pepper, rosemary, rhubarb, rue, sage, sorrel, tarragon, thyme and wormwood. Many of these, of course, were probably listed on the Assyrian clay tablets recovered from Nineveh, revealing how much interchange of medical lore there was in the Ancient World. There were also about 90 minerals, including calamine, mercury and sulfur. Passed down the generations, De Materia Medica strongly influenced Arab physicians after the fall of Rome. It was subsequently among the first books to be printed and it continued to be widely accepted as an infallible authority until the eighteenth century.10,11

Pliny the Elder

Unlike many of the prominent figures in Greek medicine, Pliny the Elder (23–79) was a Roman. He compiled an encyclopaedic work dealing with around 900 herbs, The Natural History. Written with somewhat less rigour and care for detail than was De Materia Medica, it reflected the use of folk medicines by those who could not afford the services of a physician and was more readable than the herbal of Dioscorides. Pliny was less selective than Dioscorides and, like Theophrastus, had no hesitation in incorporating magical and superstitious material. The use of Latin later facilitated its being copied in monasteries, ensuring that its magical and superstitious elements would be perpetuated for many generations thereafter. For over 500 years after the fall of Rome it was the principal source of medical knowledge in many parts of Europe.

Galen

The most influential of all the Greek physicians was unquestionably Galen (AD 129–199). Born in Pergamon, he began his study of medicine at nearby Smyrna, completing his training 12 years later in Alexandria before moving to Rome in AD 162. He achieved considerable fame there as a clinician and his amassed wealth allowed him to become a prolific writer whose output dwarfed that of all other ancient writers. His collected work in 20 volumes is entitled Opera Omnia. This enshrines literally all the medical knowledge of his day. In it, Galen expressed admiration of Hippocrates and Dioscorides, although distorting their ideas to suit himself. Specifically, he taught that imbalance of the four humours was the cause of disease, but went much further by offering the dogmatic view that it should be possible to correct humoral imbalances, not only by supplementing deficient qualities with herbs possessing similar properties but also by administering other herbs that had opposing qualities. He expressed this succinctly in his maxim ‘contraria contrariis curantur’ (opposites cure opposites). This was the origin of a medical system that beguiled physicians for the best part of two millennia. It was the antithesis of what modern medicine strives to do by identifying the causes of disease and addressing them directly by experimental investigation, supported by controlled clinical trials to evaluate efficacy of drugs.

Galen’s approach to therapy also involved polypharmacy, numerous plants being compounded in complex formulations that became known as galenicals. His enthusiasm for the Theriac of Andromachus illustrates this. He argued that the body was capable of selecting whichever ingredient would correct the humoral imbalance. Whenever the followers of Galen encountered failure of a remedy to cure, they exercised their minds trying to decide which extra
ingredients to incorporate in the hope that they might possess appropriate properties to correct specific qualities that were deemed to be either in excess or else deficient.

Galen claimed that nature acted with perfect wisdom and the body was but the instrument of the soul. This appealed to the Church and reinforced the authority of Galen, making it difficult for anyone to question his teachings. The credence given to his writings exerted a stultifying influence on therapeutic practice, not only while the Roman Empire flourished but right up until comparatively recent times. So pervasive was his influence that until pathology developed into a mature science in the nineteenth century, when it was bolstered by Pasteur’s germ theory of disease, it was generally accepted that illness consisted of a disruption of the constitution. This severely impeded the search for external causes of disease and consequently the development of effective drugs.

Many of the extant works of Galen are available on-line from the Bibliothe`que Interuniversitaire de Médecine.12 They are also available in print.13

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The origins of Baghdad as the leading medical centre of the Muslim world in the ninth century are obscure, but it has often been claimed that in the year 765 Jirjis Bakhtyishu’, the head of a prominent medical school in Jundishapur (situated about 300 miles to the south-east of Baghdad), was summoned to the capital to treat Caliph al-Mansur for dyspepsia. The likelihood of Bakhtyishu’ covering such a distance over rugged terrain in time to treat such a minor, self-limiting complaint must be questioned, but the successful outcome is said to have resulted in his remaining in the city for many years. His son, Jibra’i ibn Bakhtishu’, is then believed to have become one of its leading physicians and founded its first hospital in 805 at the request of the fifth Abbasid Caliph, Harun al-Rashid. There is, however, no convincing evidence that a leading medical school ever existed in Jundishapur. The Bakhtyishu’ family, one of two leading Nestorian medical families in Baghdad over a period of nearly three centuries, may have created the story.

A more probable account of the emergence of Baghdad as a medical centre involves Nestorian Christians. Nestorians believed that Jesus was two persons, one divine and one human. Their stronghold had been Edessa, situated in what is now south-eastern Turkey, near the border with Iran. The Nestorian Church spread out from this centre of Syrian culture to form its own traditions. Its scholars translated the Hebrew Bible into their native language, Syriac, with ease as it was similar to Aramaic, itself not greatly unlike Hebrew. They also studied Greek so as to translate the Septuagint, a Greek rendition of the Hebrew Bible compiled by 70 Jewish scholars in Alexandria in 132 BC. Both versions of the Bible were similar, but in order to address theological issues their differences were compared in Syriac, the native language of the Nestorians. When the Byzantine Emperor Zeno expelled the Nestorians from Edessa in 489 for their heretical views, they fled eastwards, taking with them knowledge of the Greek language and literature. After Baghdad was established some settled there and so, too, did Arab, Jewish, Alexandrian and Indian scholars, all contributing to the intellectual milieu of the new city.

In the ninth century, Baghdad became the focus for a revival of humoral medicine, which elsewhere was receding as classical culture declined and medicine became compromised by the Christian belief in disease being a punishment for sin. Several factors can account for the revival, not the least of which was the presence in Baghdad of Nestorians and other immigrants who were able to translate into Arabic the classical medical texts from either Greek or Syriac versions. Arabic had replaced the now neglected Greek as a universal language of scholarship. Also, the prosperity of the city favoured the existence of an educated elite who could afford the teaching and books required to study medicine. Finally, as a result of the Muslim territorial expansion that had occurred there was access to classical texts stored in the monasteries of Egypt, Syria and Iraq that could be borrowed, purchased or simply taken as the spoils of war.¹
HUNAYN IBN ISHAQ

While translation of classical texts had already begun during the caliphate of Harun al-Rashid, it was boosted by the desire of his son Ma’mun to support the Mu’tazila clergy who were seeking a rational basis for Islamic belief that could challenge that of Christianity and other religions through the assimilation of acceptable ideas and opinions enunciated elsewhere. A year before his death in 833, Ma’mun established the Bayt al-Hikma (House of Wisdom) in Baghdad, where scholars gathered to collect and translate into Arabic not only classical texts but any others that were considered to contribute to the development of Islamic culture. It was here that the Nestorian translators came to the fore, the most notable being Hunayn ibn Ishaq (known in mediaeval Europe as Johannitius). He had been a pupil of Yuhanna ibn Masawayh (777–857), a member of a prominent Nestorian family who was physician to four Caliphs of Baghdad, and was to become well known in the West through his treatises on various aspects of medicine that were translated into Latin with the name Mesue as the author.

Established as a translator of the Hebrew Bible into Arabic, Hunayn together with his associates tirelessly sought far and wide to recover a vast number of medical and other texts, especially those written by Galen, and then translate them with great care and attention to detail. This hectic activity continued unabated after the death of Ma’mun until Caliph Mutawakkil, an opponent of the Mu’tazila, came to power in 847. A steady decline in the output of the Bayt al-Hikma followed, and eventually lone translators were left to work unassisted in that once great institution. However, the legacy of the Bayt al-Hikma remains with us in the hundreds of texts that were translated there into Arabic and thereby saved. Its collection of so many works by Galen also resulted in the spread of humoral medicine throughout the expanding Muslim world and planted the seed for the further development of Arabian medicine. There were many who took up this cause, resulting in a massive literature being compiled over many centuries by a large number of writers, of whom only a few can be mentioned here. From the tenth century onwards, the complexity of this stimulated the writing of encyclopaedic tomes that charted the path for physicians to follow.1

One of the consequences of the revival of Galenic medicine during the caliphate of Ma’mun was a demand for complex formulations that required specialised skills for their manufacture. Initially this resulted in the development of a new profession, that of the apothecary – later to be described as a pharmacist – and soon these licensed apothecaries opened shops in Baghdad which were under the watch of inspectors employed by the state.

ABU BAKR AL-RAZI

Among the greatest of the physicians who practised in Baghdad was Abu Bakr al-Razi, or, simply, al-Razi (known in the West as Rhazes), a Persian who died in the year 925. He was not only the director of a hospital in the city but was also an eminent philosopher and author of around 200 books on a diverse range of subjects. His medical writing included two major works, Kitab al-Mansuri fi al-tibb (The Book of Medicine for Mansur) and Kitab al-Hawi fi al-tibb (The Comprehensive Book on Medicine). The former consisted of ten volumes covering a wide range of specialities from both a theoretical and practice-based viewpoint, the use of drugs being covered in the first four volumes. Gerardo de Cremona (1114–1187) translated the entire work into Latin as the Liber Almansoris in Spain in the late twelfth century. The second of two major works by al-Razi was compiled by his students after his death from his extensive files and was published in 20 volumes that then constituted the largest collection of medical writings, including material from Greek, Hebrew, Syriac and Arabic sources. It is noteworthy that al-Razi recommended the testing of drugs on animals before exposing humans to them. His caring attitude towards his patients even extended to his introduction of opium to sedate those upon whom he practised surgery.
ABU ALI AL-HUSSAIN IBN ABDALLAH IBN SINA

Al-Razi was surpassed in his influence by yet another Persian philosopher who both practised and wrote prolifically about medicine, namely Abu Ali al-Hussain ibn Abdallah ibn Sina (980–1037), generally known in the West as Avicenna. He was a devout follower of Galen, whose ideas he expounded and developed further. His fame was in large part due to the encyclopaedic al-Qanun fi at-tibb (The Canon of Medicine), of which the Latin translation by Gerardo de Cremona became adopted as the standard text for medical students throughout Europe until the eighteenth century. In this magnum opus of nearly one million words, ibn Sina merged Greek and Arab traditions to encompass all that he had learned from diligent reading of ancient manuscripts, and then he embellished his book with his own clinical experience. He also adopted an Aristotelian approach that resulted in Galen’s complex ideas being lucidly presented for the first time. In the second of the five main sections, ibn Sina described the uses and efficacy of 760 drugs arranged alphabetically, these being his preferred drugs. Thousands of others favoured throughout the Arab world were rejected. Such was the influence of this authoritative book that it ensured the perpetuation of Galenic medicine for many centuries, during which time no one dared question the teachings of ibn Sina. He undoubtedly had a brilliant mind and was one of the greatest physicians of all time, but his influence delayed the demise of an ancient system of medicine that in retrospect can be seen to have ill served those who sought benefit from it. If a genius of the stature of ibn Sina could have embraced humoral medicine so wholeheartedly, it is little wonder that it still persists to this day in the Unani medicine of India and strongly influences the use of contemporary herbal products favoured by proponents of complementary medicine.

ABU AL-QASIM AL-ZAHRAWI

It was not just in the East that Arabic culture thrived after the Muslim conquests. Under the enlightened rule of Abd-er-Rahaman III, Córdoba, and in particular the suburb of the Umayyad royal palace, rivalled Baghdad in its grandeur until its conquest by the Berbers a century later. With a library of 400,000 manuscripts, it became a great centre of learning and scholarship where physicians could study the ancient classics. The most illustrious of them was Abu al-Qasim al-Zahrawi (936–1013), often known in the West through his Latinised name Albucasis. He was the author of one of the greatest encyclopaedic medical texts ever written, al-Tasrif li-man ’ajaza ‘an al-ta’lif (The Guide for Him Who Cannot Compose . . .). This masterpiece was written a few years before the Canon of ibn Sina at the time when Arabian medicine was approaching its zenith. It records the state of medical and pharmaceutical knowledge 1000 years ago, but unfortunately only parts were ever translated. The last of its 30 volumes, an illustrated manual of surgery, was translated into Latin by Gerardo de Cremona and subsequently printed in Venice in 1497. Under the title De Chirurgia Parva, it became widely studied throughout European medical schools during the Middle Ages, resulting in its author becoming best known as a surgeon. The rest of al-Tasrif, however, contained a vast amount of chemical, pharmaceutical and therapeutic information. The 28th volume was translated into Latin in 1288 by Simon of Genoa and printed in Venice in 1471, becoming known as Liber Servitoris de Praeparatione Medicinarum Simplicium, or simply Liber Servitoris. It was relied on by many generations of European apothecaries as a first-hand source of information on pharmaceutical processes. That a physician wrote it might seem surprising, but can readily be explained. The development of an independent profession of apothecaries that took place in Baghdad was not paralleled in Spain; hence physicians there were left to compound their own medicines. As there was no reliable source of written information about such matters, possibly because the apothecaries in Baghdad preferred to
keep their knowledge secret, al-Zahrawi set about filling the void in the 28th volume of *al-Tasrif*.

The 28th volume is in three parts. The first deals with how to obtain, store, grind and reduce or oxidise a variety of minerals used in medicine, including alum (aluminium sulfate), copper, galena (lead sulfide), iron, stibnite (antimony sulfide) and vitriols (natural sulfates). The preparation of the oxide and carbonates of lead are fully described together with the purification of ceruse, a white lead carbonate found near Córdoba and which was used as a cosmetic. The preparation of verdigis (basic copper acetate) from copper is likewise discussed. The text also expounds on the roasting (calcinations) of mercury and of arsenic in a furnace to form their oxides, and also details a variety of chemical processes such as the purification of tutty, a sublimate of zinc oxide that adhered to the flues of furnaces in which zinc ores were smelted, and the preparation of sal ammoniac (ammonium chloride) from igneous rocks.

As Arab physicians were strongly influenced by Galen, it might seem surprising to encounter such detailed information on the preparation and uses of minerals, about which Galen was certainly far from enthusiastic. It has been suggested that this happened because of a strong association between Arab and Indian medicine which arose from the presence of Indian physicians in late eighth century Baghdad. In India, links between medicine and alchemy had been developed in the fourth and fifth centuries and subsequently many Arab physicians dabbled in it, thereby ensuring a continuing supply of minerals for medicinal purposes. Troubled by a hot, dry climate and frequent sandstorms, their patients often developed eye and skin diseases for which the application of soothing ointments containing sulfur and mercury became almost routine. So far as the internal administration of minerals was concerned, however, restraint seems to have been exercised.

The second part of the 28th volume, in contrast, is a treasure trove of pharmacognosy that is concerned with the correct manner of handling plant products for medicinal application, with an emphasis on the importance of drying and storage techniques. Examples include acacia, aloes, cardamom, colocynth, fleawort, fumitory, galbanum, lily, liquorice root, lycium, mandrake, opium, scammony, sandalwood, spurge, squill and wormwood. The natural habitat of each herb is described, together with a description of the plant and how to select the appropriate component and at what season. The expression of juices and collection of gums is covered, as is the filtering of decoctions and other liquid preparations. There is information about preparing oils, vinegar, aromatic waters, amber, coral, turpentine and so forth. A wide variety of pharmaceutical techniques familiar to pharmacists of recent generations are also expounded upon in this part of the book.

The last section of the 28th volume deals with products obtained from animal sources. Guidance is provided on the collection of shells, nails, hooves, bones, fat, blood, milk, urine, snake skins, scorpions and other similar necessities of medieval pharmacy. As in the other two sections, a variety of pharmaceutical processes are again described, complete with illustrations of the apparatus required.

Throughout the ages there were many who believed that the *Liber Servitoris* represented the complete pharmaceutical writings of al-Zahrawi. That view was entirely mistaken. The 3rd volume of *al-Tasrif*, for example, gives a detailed account of the compounding and storage of concoctions, while the 10th volume ends with a discussion on preparations of seeds and nuts to treat gastrointestinal complaints. Similarly, the 15th volume encompasses the production of conserves prepared from fresh or dried herbs, even going into the details of incorporating aromatic spices to enhance their acceptance. The 16th volume concentrates on the preparation and storage of powders, whereas the 18th volume considers a more diverse range of products, including astringent and dusting powders for application to wounds, ear drops, effervescent powders, fumigations, gargles and incenses. The 20th volume covers preparations for the eye, including compresses, drops, washes, lotions and ointments, whereas the 21st describes applications for the mouth and throat, such as dentifrices and lozenges. Cough remedies
appear in the next volume, while ointments prepared from animal fat are to be found principally in the 24th volume. Here, as elsewhere in the *al-Tasrif*, al-Zahrawi clearly acknowledges his indebtedness to previous authorities such as Galen and al-Razi. The 25th volume deals with the manufacture of fats and oils extracted from plants, which were more than just ancient products as far as al-Zahrawi was concerned; he was adamant that they remained of major importance as medicines. Listed among the sources of what he called ‘adhan’ are apricot, castor seeds, hazelnuts, henbane, linseed, peach, poppy seeds, radish, sesame nuts, sweet almonds, walnut, wheat and many more. Not only does al-Zahrawi describe the various manufacturing processes of these but he also expands upon their role in medicine, complementing this with detailed formulae and dosage. The 29th volume was of particular value to the apothecary or pharmacist because of the multilingual lists of drug names and synonyms that appeared in it, accompanied by details of their stability.

The *al-Tasrif*, however, was written for physicians, not apothecaries; hence much of the text was concerned with the use of drugs. This was covered strictly within the confines of humoral medicine, with all products evaluated in terms of their qualities, namely hot, cold, dry or wet. As an admirer of Galen, its author did not hesitate to compound a variety of ingredients together in the expectation that the body will select the most appropriate one. This was carried to an extreme in the 4th volume where he elaborated upon the seven-stage preparation and the 84 ingredients of the famous antidote known as the Great Theriac. It incorporated balm, black pepper, cinnamon, ginger, glycyrrhiza, honey, mushroom, opium, psillium, rhubarb, saffron and viper flesh. Similar multi-ingredient concoctions were developed by others to protect against stings and the bite of venomous animals. Under the name ‘theriaka’ these worthless herbal preparations acquired the reputation of being panaceas against all ills and, as such, were so highly valued that their content was kept secret. From mediaeval times until the nineteenth century, leading European civic authorities were responsible for providing their own locally produced theriacs to their populace. Perhaps this accounts for the continuing faith in herbal remedies among the general population. Consider, for example, garlic – a panacea first proposed as a cheap substitute for theriac in the seventeenth century by Nicholas Culpepper under the name of ‘treacle’, a corruption of ‘theriac’.

There were other products that rivalled the theriacs. The 5th volume of *al-Tasrif* described bitter medicines called ‘hieras’. Originally introduced by the Greeks and used in their temples, the most popular of these was hiera picra (the sacred bitters). Containing a variety of bitter herbs such as aloes, cinnamon and colocynth, the hieras gained in reputation what they lacked in efficacy, and at times they, too, acquired the status of panaceas for all ills.

There were more mundane products as well in *al-Tasrif*. For example, the 6th volume discussed purgatives, selecting principally those bitter ones that could expel black bile and yellow bile. This included such powerful agents as aloes, colocynth, euphorbium, scammony and squill. These acted by irritating the bowel, so it is just as well that al-Zahrawi recommended that they should be reserved for healthier patients, with smaller doses being more appropriate for certain cases! More pleasant tasting purgatives were discussed separately in the 8th volume, these being considered suitable for those who vomited if prescribed a bitter agent. The 10th volume was similar, describing a variety of seeds and nuts that could be administered as purgatives. The apparently disproportionate coverage of purgatives is merely a reflection of the limited range of procedures for ridding the body of excess humours.

As would be expected in a system of medicine that claimed all illness was due to humoral imbalance, al-Zahrawi recommended specific herbs on the basis that they could correct this and thereby heal a wide variety of ailments associated with that imbalance. This remains a common feature in contemporary herbal medicine. There was, in addition, the alternative of treating a single disease with a variety of herbs thought to have similar corrective effects on the perceived imbalance. Thus, in his 9th volume, al-Zahrawi advocated hot, aromatic drugs to treat heart disease; this was considered to arise from an excess of phlegm. The discerning
observer may, of course, complain that these two approaches gave carte blanche to the incompetent healer to administer just about anything at all. This is evident in the 12th volume, which covers remedies for both obesity and for lack of weight, for either excess or deficiency of semen, for over- or under-production of mother’s milk and, inevitably, aphrodisiacs.

The *al-Tasrif* was a remarkable work which al-Zahrawi himself readily acknowledged leant heavily on the contributions of generations of medical writers from the time of Dioscorides and Galen onwards. The *al-Tasrif* now exists only as volumes and fragments scattered around the world in different libraries, and until the publication of the study by Hamarneh and Sonnedecker and subsequent publications by Hamarneh only the *Liber Servitoris* and the surgical sections were generally known in the West. The account given here is an attempt to distil some of the essence of their brilliant work. However much we may admire the humanity, scholarship and surgical skills of al-Zahrawi and his fellow physicians, there can be no hiding from the fact that they remained uncritical of the medicine of Galen and consequently their contribution to history was the perpetuation of an unsound system of medicine that ultimately failed to discover safe, effective medicines. Instead, Arab physicians continued to introduce worthless and sometimes harmful products, which is best illustrated by a text of the thirteenth century Andalusian ibn al-Baytar which listed over 3000 plants, animals and minerals. However, in their development of pharmacy as a highly skilled profession, the apothecaries of Baghdad unquestionably played a decisive role.

**ARABIAN MEDICINE IN EUROPE**

After the collapse of the Western Roman Empire in the fifth century, the ancient Greek heritage that it had nourished was gradually lost to most of Europe. There was, however, a notable exception in Southern Italy as this territory remained part of the Eastern Empire of Byzantium even after the invasion of Italy by the Lombards in 568. Greek culture still persisted when Benedictine monks established a hospital in the west coast city of Salerno in the late seventh century. A medical school gradually developed there, probably in the ninth or tenth century. It was clearly a secular institution, for both women and Jews taught Greek medicine alongside priests, while some of the staff were even married. Furthermore, the Jewish and Christian practice of one master teaching one pupil was abandoned in favour of the entire staff collaborating in the teaching of students.

The Muslim conquest of Sicily in the year 827 inevitably had an influence on medical practice in Salerno, especially when Arab communities began to settle nearby. However, the arrival in Salerno of a scholar born in Tunis and who had travelled throughout much of the Arab world was the defining point for the introduction of Arabian medicine in Salerno. Generally known as Constantinos Africanus (c. 1020–1087), he translated a vast number of Arabic texts into Latin, including some that were much more reliable translations of Ancient Greek texts than those that had hitherto been available in Salerno – despite these having originally been translated directly into Latin. Most of his translations were made at the nearby Benedictine monastery of Monte Casino where he resided after converting to Christianity. The impact of his work was to strengthen the understanding of Greek medicine through the new insights his translations afforded of the works of its leading proponents. He also introduced a familiarity with early Arabian medicine into the school. There was consequently some movement from a preoccupation with Hippocratic medicine, with its emphasis on diet and hygiene coupled with restraint in the use of drugs and a reliance on preparations of single drugs, towards the interventionist polypharmacy of Galen so much favoured by Arab physicians.

In 1140, Nicolas of Salerno produced his *Antidotarium* of 139 compound formulas that featured Galenical remedies for opposing the excess humours that were believed to cause disease. It also included a table of weights and measures that was later to become known as the...
apothecaries’ system after the book became the first drug formulary to be set in print in a
Venice edition of 1471. However, Hippocratic teaching still prevailed at Salerno as a few years
later Matthaeus Platearius of Naples wrote his Circa Instans, so named on account of its
opening words. It was loosely based on a Latin translation of Dioscorides, being quite
different to the Antidotarium insofar as it described 273 simple herbs that were used in the
medical school at Salerno in the Hippocratic manner. These were arranged alphabetically, an
arrangement first employed by Galen. The Circa Instans strongly influenced Le Grand Herbill
printed in Paris around 1520, and a translation of it was printed in England in 1526 under the
title The Grete Herball.

By the eleventh century the reputation of Salerno had spread throughout Europe, and
pupils were drawn from far and wide. However, in 1139 the Lateran Council banned Christian
clergy and monks from practising medicine for the practical reason that increasing demand for
their services was diverting them from their ecclesiastical duties. This eventually led to a
greater need for schools such as Salerno; hence the following century saw the establishment of
rival institutions at Montpellier, and in the new universities of Paris, Oxford, Padua and
Bologna. These were soon to eclipse Salerno, hitherto the bastion of Hippocratic medicine in
Europe. For the next three or four centuries it was the polypharmacy of Galen enshrined in
Arabic medicine that was to become fashionable. At Salerno, preoccupation with Greek
medicine kept its scholars from seizing the opportunity of translating the works of a new
generation of Arab physicians. Translating al-Razi, ibn Sina, Al-Zahrawi and their
contemporaries was left to others.

TOLEDO

Just as Greek medicine was revived in the Arab world after the fall of the Western Roman
Empire, through a twist of history a similar revival of Arabian medicine ensued in Christian
Europe after the Arabs were driven out of Spain. Toledo fell to the Christians in 1085,
Córdoba in 1236 and finally Granada in 1492.

Reference has already been made to the translation of al-Razi’s Kitab al-Mansuri fi al-tibb,
ibn Sina’s al-Qanun and the 30th volume of al-Zahrawi’s al-Tasrif into Latin in the late twelfth
century by Gerardo de Cremona. It was largely through the efforts of this prolific translator
that Christian Europe obtained access to the major Arabic medical texts that had been
neglected at Salerno. He had settled in Toledo, a cosmopolitan centre in Central Spain where
Arabs, Christians and Jews freely intermingled. It was here that most of the manuscripts from
the great library of Córdoba had been brought for safekeeping after its conquest and looting
by the Berbers in 1013. Gerardo was able to translate more than 60 of the major works in this
collection before his death in 1185.

LATIN TRANSLATIONS OF ARABIC TEXTS

Christian Europe had lost access to Greek medical texts during the fifth to twelfth centuries
due to the supremacy of Latin in the Church, which meant that it became the language of
intellectual discourse. Ancient manuscripts were copied and preserved in the monasteries, but
were often restricted to those written in Latin as most monks knew no Greek. However, the
only major medical text in Latin was the unreliable work of Pliny.

The motivation for looking after the sick may have reflected the highest ideals of
Christianity, but lack of access to Greek medical texts meant that the basis of medical practice
was considerably restricted until these became available in the twelfth century in the Latin
translations of Arabic renditions of the Greek texts. When this finally happened, these were
eagerly sought in the rapidly growing number of hospitals then being established throughout
Europe by Benedictine monks and others. They were also sought by the newly designated
apothecaries, so named in Venice because they were responsible for storage of the many herbs imported from Arab countries and elsewhere. The physicians and students in the new medical schools also required access to the texts. In short, there was an insatiable demand for the translations of Arabic texts, one that could not be met until Gutenberg introduced the printing press at Mainz in Germany in the mid-fifteenth century.

Due to the inferiority of mediaeval education compared with classical times, the clarity and apparent logic of ibn Sina was greatly preferred to the difficult and sometimes convoluted writings of the man who had inspired him, Galen. A thorough knowledge of the *Canon of Medicine* soon became a *sine qua non* for a medical career and Arabian medicine became fashionable throughout Europe. Few challenged its basis, with no other significant medical developments taking place. A notable exception was the Italian poet Francesco Petrarca (1304–1374) who argued that, because medicine was a practical art, those physicians who applied the methods of philosophical enquiry to it failed to cure their patients. He was proved correct by the onset of the bubonic plague against which all the ancient nostrums were to prove utterly worthless.

REFERENCES

With the development of the printing press by Johann Gutenberg in the mid-fifteenth century, the medicine of Galen and ibn Sina attained a new lease of life, but initially not through the publication of their books. After the collapse of the Western Roman Empire there had been a continuing decline in scholarship throughout Europe. Consequently, major medical texts were beyond the comprehension of many. As a result, there was a demand for simplified herbals and formularies listing drugs and their uses, exemplified in the popularity of texts such as the *Herbal of Apuleius Platonicus*, written by an unknown fourth or fifth century author, originally in Greek. It was little more than a recipe book, full of spells and pagan incantations to the earth goddess, yet its Latin translation circulated widely throughout mediaeval Europe. It was the first herbal to be printed when a version based on a manuscript from the monastery at Monte Casino in Italy was produced in Rome in 1481, bound together with a sixth century Latin translation of Dioscorides.

Gutenberg’s partner Peter Schoeffer printed editions of two herbals in 1484 and 1485, respectively. The first was compiled from the works of earlier writers and was crudely illustrated. Like other early printed works it did not have a title page, so it has become known as either *The Latin Herbal* or *Herbarius Moguntinus* (*The Mainz Herbal*). It was intended for local consumption, for it featured only herbs cultivated in Germany. There was still no awareness of the regional variation in plants, but once herbals began being printed in different locations around Europe this gradually became apparent and stimulated interest in botanical investigation among members of the medical profession who recognised that accurate plant identification was essential if their patients were to receive the intended medication.

Furthermore, the wide circulation of printed herbals exposed errors and inconsistencies in the identification of plants, not only in the translations of ancient texts but also in the newly produced herbals. The correction of these errors had the unintended merit of introducing a degree of standardisation of medicines for the first time in history.

Encouraged by the success of his first herbal, Schoeffer turned his hand to producing a text in German, *The German Herbal* (again known by a variety of names including *Herbarius zu Teutsch, Gart der Gesundheit*). It may have been compiled by von Cube, the city physician of Frankfurt, and was a far more significant work than Schoeffer’s earlier one, covering 435 plants and animal products as well. The crude illustrations, 65 of which were drawn from nature, were woodcuts. For half a century they were frequently copied despite the improbability of the plants being identified. The quality of illustrations did not improve until Otto Brunfels (1488–1534), a Lutheran preacher born in Mainz and who had turned to a career in medicine, published *Herbarium Vivae Eicones* in 1530. This had 135 illustrations prepared from woodcuts by Hans Weiditz, an artist who broke with convention by copying the plants in great detail as he made his engravings. This unique approach allowed physicians to recognise the plants mentioned in the book. Brunfels also encouraged Hieronymus Bock (1498–1554) to publish a similarly illustrated herbal in German in 1546, his *Kreuterbuch*, which included his own observations on plants that had not hitherto been described by others. This was an early stirring of independent enquiry that was now appearing throughout Europe.
The most influential of the Lutheran herbal writers was Leonhart Fuchs (1501–1566), whose *De Historia Stirpium* was published in 1542. As professor of medicine at Tübingen, he was disturbed by the relative ignorance of herbs among his fellow physicians and produced his herbal to remedy matters. Over 500 plants were described, most of which flourished in Germany. The text was also printed in German the following year. The Flemish herbalist Rembrant Dodoens (1517–1585) was strongly influenced by Fuchs in the writing of his *Cruydeboeck*, published in 1554, and *Stirpium Historia Pemptades Sex*, published in 1583. The latter was plagiarised by John Gerard (1545–1612) and expanded in 1597 as *The Herball or Generall Historie of Plantes*. A revised edition compiled by the London apothecary Thomas Johnson in 1633 corrected many of its errors and remained highly popular until the nineteenth century.

Despite the spirit of enquiry that was in the air, the ancient works of Dioscorides were not forgotten. The recognition of the unreliability of versions of his work that had appeared in print encouraged the apothecary Valerius Cordus (1515–1544) to travel widely to collect the herbs described by the Greek master. The herbal that resulted from his endeavours, *Historia Plantarum*, was edited by the Swiss naturalist Conrad Gesner and published 17 years after the early death of Cordus.

During a visit to Nuremberg in 1542, Cordus had received 100 guilders from the City Council for his dispensing notes. The Council published these two years after his death as its *Dispensatorium*. The first official pharmacopoeia to be printed was *Nuovo Receptario*, compiled in Florence by its Guild of Apothecaries and the Medical Society in 1498. Other cities subsequently produced their own pharmacopoeias too, including Basel (1561), Augsburg (1564) and London (1618).

In 1544, the Italian medical botanist Pietro Andrea Gregorio Mattioli (1501–1577) published *Di Pedacio Dioscoride Anazarbeo Libri Cinque*, his celebrated translation of the herbal of Dioscorides, complete with a commentary. He translated it into Latin shortly afterwards as *Compendium de Plantis Omnibus*. Many editions were published, the later ones featuring large woodcuts that greatly assisted identification of the plants.

**HERBS FROM THE NEW WORLD**

The early herbals were written at the same time as the voyages of discovery were opening up new trades routes. Fernandez de Oviedo y Valdes, a Viceroy of Mexico, sent the first account of medicinal plants from the Americas to King Charles V of Spain. This was published in Toledo in 1525 as *Sumario dela Natural y General Istoria delas Indias*, but it had little impact compared to the contribution from Nicolas Monardes (1493–1588), a physician who practised in Seville, a port with a monopoly in trade with the Americas. He took full advantage of the opportunity of investigating the strange herbs that his patients frequently obtained from the captains of newly returned galleons. It was probably fear of the unknown that drove him to test these on animals before contemplating administering them to his patients. Before long, he had begun cultivating the herbs in his own garden to ensure adequate supplies. His enthusiasm for the new herbs from the Americas led to the publication of the first volume of his three-part illustrated *Historia Medicinal de Indias Occidentales* in 1565. The remaining parts were published in 1571 and 1574 and were so enthusiastically received that expanded editions and numerous translations soon appeared. An English translation was printed in 1577 under the title *Joyfull Newes Out of the Newe Founde Worlde*. The complete work mentioned products such as balsam, china root, coca, guaiac, jalap, passion flower, guava, sarsaparilla, sunflower and tobacco, all of which soon appeared in the premises of apothecaries throughout the continent.

Monardes was not the first to recommend guaiac, a resin exuded from the heartwood of the small West Indian tree known now as *Guaiacum officinale* L. This was already in wide use
against syphilis, then generally believed to have been imported into Spain on the return from the West Indies of those who sailed with Columbus. Infected Spanish colonists on observing that the indigenous population were treating the similar disease of yaws with guaiac, promptly concluded it would also cure syphilis. Their view was bolstered by the ability of large doses of guaiac to induce diuresis, expectoration, purgation and profuse sweating. For those familiar with humoral medicine, no better indication of efficacy need have been sought. They even went so far as to recommend the resin for the relief of rheumatism and gout. When guaiac failed to cure syphilis, there were other herbs from the same region they could turn to, such as china root (*Smilax chinea* L.) or sarsaparilla (*S. aristolochia*).

Nor was Monardes the first to describe tobacco, *Nicotiana tabacum* L. The Spaniards became acquainted with tobacco smoking when Columbus landed in Cuba in 1492. Hernandez de Toledo later brought the plant back to Spain because it was thought to have medicinal properties. Joan Nicot subsequently sent the seeds to France about 1560. From then until the middle of the nineteenth century, tobacco was used as an antispasmodic, often administered rectally in the belief that the smoke would rise further up the intestines than any liquid enema and so be more efficacious. Medicinal cigarettes were popular in the nineteenth century, incorporating ingredients such as belladonna, atropine, hyoscyamus, aniseed, amber or nitre. The latter was converted to nitrite on burning, which relaxed the bronchi. Particularly popular were commercial brands such as Grimaud’s Cigarettes® containing cannabis resin, Cigarettes de Joy® containing arsenic, Crevoisier’s Cigarettes® containing foxglove and Savory & Moore’s Cigarettes® containing camphor.

**CINCHONA BARK**

Two genuinely efficacious herbs from South America were introduced into Europe in the sixteenth century. The first of these was cinchona bark, *Cinchona officinalis* L. There are two accounts of how it was introduced into Europe. A fictitious version was included in a book known as the *Anastasis Corticis Peruviae*, published in 1663 by a Genoese physician called Sebastianus Badus. He claimed that 30 or 40 years earlier, during an epidemic in the city of Lima in Peru, the bark had cured the Countess of Cinchon, wife of the viceroy of the province, of a severe attack of tertian fever (malaria). In gratitude, the countess distributed the bark to sick people in the town and they were also cured. After the countess returned to Spain, the bark was used there and then taken to Rome for testing by Cardinal de Lugo in 1649. Romantic though this tale may be, a fascinating book written by Saul Jarcho has thoroughly undermined it.2

Jarcho also examined the other account of the introduction of cinchona. He found several sources for it, the principal one being a book by Gaspar Caldera de Heredia, entitled *Tribunalis Medici Illustrationes et Observationes Practicae*, published in Antwerp in 1663. In this, it was recounted that in a remote part of the Peruvian province of Quito, the Indians had to cross a river up to their necks in water and thereby became intensely cold. To stop shivering, they would drink a decoction of the bark in hot water. When Jesuit priests observed the striking effects of the bark, they asked for some of it to investigate. Reasoning by analogy, they tried the bark to relieve their own bouts of coldness and shivering caused by the intermittent fevers of malaria. Before long, this revealed to them the efficacy of the bark in relieving the disease.

Jarcho also cited the 2nd edition of the *Opera Medica* by the chief physician to Philip IV of Spain, Pedro Miguel de Heredia, who died about 1661. This 2nd edition of his book, which was not published until 1689, described the use of powdered ‘quarango’ bark by the Indians of Quito after exposure to coldness and how the Jesuits successfully exploited this in treating malaria. The details given in this account are similar to those published by Caldera de Heredia,
but the author goes on to say that in Madrid he had been shown the bark by the son of the late Count of Cinchon.

The recurring account of the use of the bark by the Indians to relieve their coldness and shivering was addressed by Jarcho, who noted that a paper on hypothermia in animals, published in 1977, described how quinine (the major active principle in the bark) stopped their shivering. This was consistent with its ability to decrease the excitability of the motor end-plate region of skeletal muscle to repetitive nerve stimulation.

Jarcho also noted that Caldera de Heredia went on to relate in his book how the news of the discovery of the bark spread throughout Peru and was carried back to Spain by Dr Juan de Vega, physician to the Count of Cinchon. Trials in Spain, coupled with further reports from Jesuits returning there from Peru, ensured the general adoption of the bark. Other similar reports of the introduction of the bark into Spain corroborate this version, including a reference to it by Badus in his *Anastasis*.

Jarcho, who cited evidence relating to dates and persons which appears to confirm the validity of the account given by Caldera de Heredia, suggested that the bark first became known to Europeans around 1630, and samples were taken to Rome by Jesuits in 1631 and 1632. Jarcho also mentioned the work of others who considered it probable that Cardinal Juan de Lugo learned of the bark from Bartolome Tafur, a Jesuit from Peru who visited Rome in 1645. The Cardinal had the bark tested, and subsequently used his own funds to obtain large amounts of it for distribution to the sick. This led to cinchona being described as pulvis cardinalis or pulvis de Lugo.

Following its acceptance in Rome, the bark was sought throughout Europe. However, it was not without its dangers, and disputes broke out over its merits. The fact that it was distributed by the Jesuits led to an element of religious bigotry adding to the confusion, and it was only the efforts of an English quack, Robert Talbor, that finally ensured Jesuits’ bark a role in medicine. Talbor was an apprentice to an apothecary in Cambridge, where the Professor of Physic, Robert Brady, had begun to prescribe the bark in 1658 during a serious outbreak of malaria. Talbor moved to London, where he treated many cases of malaria with a secret remedy. In 1672, he published a small book called *A Rational Account of the Cause and Cure of Agues*. In this he warned the public of the dangers surrounding the administration of Jesuits’ bark by the unskilled. Cunningly, he suggested that this remedy should not altogether be condemned, but nowhere did he even hint that he himself had used it! The book boosted his reputation, and King Charles II awarded him a knighthood and issued letters patent appointing Talbor as his physician in ordinary. This was done despite fierce criticism from the College of Physicians, angered at the appointment of an unqualified practitioner. The physicians of Paris and Madrid were likewise outraged when, later that same year, Talbor cured the Dauphin of France and the Queen of Spain. The following year, King Charles’ judgement was vindicated when he fell ill with tertian fever at Windsor and was cured by Talbor’s secret remedy.

Grateful for the curing of his son, Louis XIV persuaded Talbor to part with the secret of his remedy for a sum of 2000 louis d’or plus an annual pension of 2000 livres. Talbor agreed on condition that the formula would not be revealed during his lifetime. When he died in his fortieth year in 1681, Louis arranged for this to be divulged in a small volume that appeared a year later, disclosing that it consisted of large doses of Jesuits’ bark infused in wine. An English translation was published in 1682 under the title *The English Remedy: or Talbor’s Wonderful Secret for Curing Agues and Fevers*.

The revelation of the nature of Talbor’s remedy was followed by the publication in 1712 of a book known as *Therapeutice Specialis* by Francesco Torti, professor of medicine at Modena. It was written to counter the prejudiced arguments raised against the use of the bark, especially those claiming that as the drug had no purgative properties it could not be expected to cure disease! Torti provided an elaborate taxonomy of fevers in order to explain which ones would be cured by the use of cinchona bark. He distinguished the continued fevers from the
intermittent fevers. The latter, often called the ague, were milder and occurred in regular cycles that were described as tertian or quartan, depending on whether the attacks took place every third or fourth day. These were the fevers that responded to the Peruvian bark. *Therapeutica Specialis* did much to establish the efficacy of cinchona bark, particularly as it dealt with the question of which types of fever would respond. The problem here was that until the general acceptance of the germ theory of disease in the latter part of the nineteenth century, physicians considered fevers to be diseases in their own right.

It is a measure of how deeply entrenched the humoral system of medicine was that it caused so many physicians to discount the value of the first curative drug they encountered. Many people had clearly been cured by it, yet there remained widespread resistance to its use. It took the efforts of a quack to overcome this. What a terrible indictment of the physicians of that period.

As demand for the Peruvian bark grew, confusion arose over its botanical origins, especially as the colour varied in different samples. The situation was only clarified after Charles-Marie de la Condamine, while engaged in an astronomical study near Quito in 1737, investigated the origins of the barks and obtained specimens. He learned the true identity of the cinchona tree from a botanist, Joseph de Jussieu. This enabled the Swedish botanist Linnaeus to classify the genus of trees from which the barks were obtained as Cinchona, a term he invented in 1742.

**IPECACUANHA ROOT**

The second effective herb introduced from South America in the seventeenth century was ipecacuanha root, *Cephaelis ipecacuanha* Willdenow. A report about its ability to cure what is now recognised as amoebic dysentery appeared in the *Historia Naturalis Brasiliae*, published in 1648 by William Piso, a Dutch physician who had returned from Brazil. Supplies did not reach Europe until 1672, but when he recommended it to his patients in Paris, they experienced toxic effects. His partner, Jean-Adrian Helvetius, was then urged by a merchant to try freshly imported root. After conducting suitable tests, Helvetius placed placards all over Paris advertising the virtues of his secret remedy for dysentery. Louis XIV heard of the remedy and arranged for it to be tested at the Hôtel-Dieu. On the successful outcome of this trial, the king paid Helvetius 1000 louis d’or for its formula. The merchant who had urged Helvetius to test the fresh root received nothing. Ipecacuanha root remained highly popular while amoebic dysentery was a regular occurrence and was one of the first herbs from which an active principle was extracted in the early nineteenth century, in Paris.

The impact of the new herbs from the Americas was significant because they had never been described by Galen or ibn Sina, yet they were believed to be powerful drugs. For some, this began to raise doubts about the opinions of these old authorities about the discovery of new drugs.

**THE DOCTRINE OF SIGNATURES**

A form of sympathetic magic which ultimately came to be known as the doctrine of signatures was originally expressed in earlier times by actions such as wearing the skin of powerful animals in order to acquire their strength or the adoption of horns as an aid to fertility because their crescent shape is similar to either the moon, which undergoes a cycle of birth and renewal, or else to the plough, which was so important in the fertilisation of the soil. To this day, rhinoceros horn is prized in the East as an aphrodisiac. A variant of this is the account in the *Book of Genesis* which relates how Rachel, the infertile wife of the patriarch Jacob, gave birth to a son after obtaining mandrakes. As mandrake root (*Mandragora officinale*) is bifurcate, it is not difficult to envisage it resembling the shape of a human. Those who sought to profit from this herb frequently strengthened this perception by appropriate carving of the
root. Ginseng was an alternative to mandrake for the same reason. Many more examples can be found in the works of historic medical writers, but it was especially in the writings of Paracelsus that the concept was fully developed. However, Paracelsus was not an author who was easy to comprehend. It was left to one of his admirers, the German master shoemaker and theologian Jacob Böhme (1575–1624), to formalise the ideas of Paracelsus as the doctrine of signatures in his *Signatura Rerum* (*The Signature of all Things*), published posthumously in 1651. In this devotional text, Böhme asserted that the Almighty had specially marked every object to reveal its purpose. This led him to believe that there were signs in herbs that could reveal their intended medicinal use. Thereafter, all who were gullible enough to believe in this doctrine eagerly sought such signs. These could take the form of colour or shape, so that iris petals were ideal for treating bruises on account of their blue–black colouring, goldenrod could relieve jaundice because of its yellow hue, blood diseases could be treated with red herbs, lung complaints would respond to lungwort with its lung-shaped leaves, likewise liverwort for liver disease, pilewort for haemorrhoids and toothwort for toothache, while dropsy (oedema), which was diagnosed by swelling of the ankles, would respond to byrony root as this resembled a swollen foot. There were other types of signs that could be identified, such as the quacking aspen for those afflicted with palsy, plants with rough surfaces for rough skin, while plants or animals that survived for many years were evidently to be considered as aids to longevity.

The doctrine of signatures lay at the heart of the writings of Nicolas Culpeper, a healer who refused to join the London College of Physicians as he held its members in utter disdain. Influenced by the belief of Paracelsus that within every object there was some sort of volatile spirit that had counterparts both in the stars and in creations of the natural world such as plants and minerals, Culpeper added an astrological dimension to Böhme’s writings. In his *Judgement of Diseases*, Culpeper asserted that the stars had imprinted signatures upon plants. When, in 1649, he had the impunity to translate the College of Physicians’ *Pharmacopoeia Londinensis* from Latin into English, Culpeper intertwined astrological concepts with the original text. At that time, astrology was universally held to be valid, its foundations having been firmly laid in many of the admired Arabic texts. It was employed in diagnosing disease, determining the most appropriate time to administer medicines and even in the preparation of medicines.

Culpeper wrote the best-known English herbal of his time, published in 1653 under the title *The English Physician*. In it he discussed 369 medicinal plants, and for each one he described the astral influence that governed its virtues. For example, on cowslip he wrote, ‘Venus lays claim to this herb as her own, and it is under the influence of Aries’, dandelion ‘...is under the dominion of Jupiter’ and fenugreek ‘...is under the influence of Mercury’. The impact of this book gradually waned during the next hundred years or so, but did not ever completely disappear. In an 1826 edition, the editor wrote: ‘Hereby you see what reason may be given for medicines, and also what necessity there is for every physician to be an astrologian.’ Many modern herbal publications for the mass market are still littered with astrological material lifted straight from Culpeper. The more serious herbals have somehow managed to extricate themselves from it, but in the public mind there still lingers the fond notion that herbs exist for the benefit of mankind and, as such, are providers of medicines to cure our ills.

**SCIENTIFIC INVESTIGATION**

The popularity of herbals from the late fifteenth century onwards resulted in their becoming the prime source of information about drug therapy rather than the longer treatises by Galen and ibn Sina. This not only contributed to a reduction in the influence of these and other ancient writers but encouraged physicians and apothecaries to conduct and publish their own
investigations. However, it was not until the late eighteenth century that these began to adopt what would now be recognised as a scientific approach.

James Lind

The Scottish naval surgeon James Lind is remembered as the first man in medical history to conduct a controlled clinical trial when he proved that drinking lemon juice could cure scurvy. In May 1747, he tested a variety of reputed remedies on 12 scurbutic sailors quartered in the sick bay of HMS Salisbury. Two of them were restricted to a control diet, but each of the others was additionally given one of the substances under trial. Both sailors who had been provided with two oranges and a lemon every day made a speedy recovery, one of them being fit for shipboard duties within six days. The only others to show any signs of recovery were those who had been given cider. Lind observed no improvement in the condition of the sailors who had received oil of vitriol (dilute sulfuric acid), vinegar, an electuary of garlic, myrrh, mustard and other ingredients, sea water or solely the control diet. He drew the obvious conclusions, which were duly acted upon by Captain James Cook on his second voyage round the world. Cook was at sea for three years, yet not a single member of his crew died from scurvy thanks to adequate provision of lemon juice, as well as fresh fruit and vegetables. Disappointingly, it was not until 1795 that the Admiralty finally agreed to a regular issue of lemon juice on British ships. The effects of this action were dramatic; whereas in 1780 there had been 1457 cases of scurvy admitted to Haslar naval hospital, only two admissions took place between 1806 and 1810. The situation then deteriorated for over a century until it was discovered that cheaper lime juice which had replaced lemon juice had only about a quarter of its antiscorbutic activity.

Anton von Störck

The prohibitive cost of the services of medical practitioners meant that folk and domestic remedies continued to be relied upon throughout the eighteenth century, the reputation of the more popular ones spreading by word of mouth. The first to conduct an examination of such medicines in a scientific manner was Baron Anton von Störck (1731–1803), a physician who began life as an orphan in the poorhouse, yet through his efforts as a physician rose to the ranks of the aristocracy in Vienna and became a confidant of the Empress Maria Theresa. He had been a pupil of Gerard van Swieten (1700–1772), who had studied under Boerhaave in Leiden before establishing the Vienna medical school.

In order to determine the safe dose of folk medicines that had previously been considered too poisonous for therapeutic application, Störck first conducted animal experiments and then tested the herbs on himself. He finally administered small doses to patients, which he gradually increased on a sliding scale until he observed beneficial effects. Störck began by investigating the deadly poison hemlock (Conium maculatum L.), the Athenian state poison that claimed the life of Socrates. He reported that it had a beneficial effect in cancer but this claim remained controversial until rejected at the end of the nineteenth century. His findings were published in 1761 in Latin and translated into English the following year as Essay on Hemlock. He next investigated thorn apple (Datura stramonium L.), henbane (Hyoscyamus niger L.) and monkshood (Aconitum napellus L.), which is also known as aconite. The latter he established as having both diaphoretic and diuretic properties, whereas the first two were said to be of value in mental disorders. As a result of these and other investigations by Störck, several neglected herbs were accepted into mainstream medicine, including monkshood, thorn apple, henbane and also colchicum corm (Colchicum autumnale L.) as a remedy for dropsy (oedema) and pulmonary effusions. Colchicum was originally found growing in the Colchis Region of Asia Minor, being known today as the meadow saffron. It was classified by Dioscorides as a
highly poisonous plant which caused death by choking, yet the most influential of the Byzantine physicians, Alexander of Tralles, recommended it in the sixth century for ‘podagra’, an opinion endorsed by ibn Sina. The German botanist Hieronymous Tragus mentioned in the 1552 edition of his Kreuterbuch that Arabian physicians employed this root in cases of gout and rheumatism. Despite this, colchicum went out of fashion among physicians, but it remained in favour among country folk. Its misuse earned it the nickname ‘colchicum perniciosum’ within the medical profession.

Nicolas Husson, an officer in the French army, in 1783 introduced a secret remedy known as Eau Médicinale, which acquired a considerable reputation for, among many other things, alleviating the pain and inflammation of gout. Eau Médicinale was brought to England in 1808, soon being rivalled by similar formulations such as Wilson’s Tincture and Reynold’s Specific. The formula of Wilson’s Tincture was a closely kept secret until it was revealed in 1818 by William Henry Williams who, by then recommending the administration of colchicum for gout, ensured the place of the plant in the treatment of that painful ailment. In the middle of the nineteenth century, a classic work on the administration of colchicum for gout was published.

William Withering

Foxglove (Digitalis purpurea L.) was another folk remedy introduced into medicine in the eighteenth century. It grew throughout Europe, originally being known as the folksglove on account of the shape of its flowers. Both this and the colour are revealed by the systematic name, which is derived from one given to it in 1539 by Hieronymus Bock. He explained that ‘digitalis’ was an allusion to the German word fingerhut (finger stall), since the blossoms resembled the fingers of a glove. Foxglove has a long history of folk use, being known to Ancient Celtic tribes. The Myddvai, the family of thirteenth century Welsh healers, applied it to the body by inunction to relieve headache, abscesses and cancerous growths. It was also listed among herbs used by Edward III of England (1327–1377).

In 1775, the English physician and botanist William Withering (1741–1799) was asked his opinion of a herbal tea for the relief of dropsy, a condition marked by accumulation of body fluid due to right heart failure. The cause was then unknown, so the aim of treatment was to expel the excess body fluid with herbs. Withering realised that among the 20 or so ingredients in the herbal tea, it was the foxglove that was most likely to be causing the violent vomiting and purging. During the next nine years, Withering treated 158 patients with it, of whom about two-thirds responded favourably. In 1785, he wrote his treatise entitled An Account of the Foxglove, and Some of its Medical Uses: with Practical Remarks on Dropsy, and Other Diseases. This described how to determine the correct dosage, which was highly relevant since foxglove was a potent poison that was ineffective unless administered at near the toxic dose level. Like Störeck, Withering took care to standardise the doses he used, going even further by determining the correct dosage for each patient. Withering also discussed different ways of preparing foxglove, preferring the use of powdered leaves. However, he did not realise that its stimulant action on the heart was responsible for its beneficial role in dropsy. Indeed, it was not until 1799, the year of his death, that Ferriar suggested that the increased urinary output was of secondary importance compared with the power of foxglove to reduce the pulse rate. Because of the lack of a clear understanding of how it was able to affect dropsy, foxglove was rarely used as a cardiac stimulant during the nineteenth century. Although the German pharmacologist Ludwig Traube revealed the stimulating effect of foxglove on heart muscle in 1850, it was not until 1901 that a clear understanding of the effects emerged through the development of the polygraph by the Scottish physician James Mackenzie and the electrocardiograph by the Dutch physician Willem Einthoven. Subsequent investigations elucidated the correct indications for the use of digitalis, namely in atrial fibrillation, where the
co-ordination of the contraction of individual muscle fibres is so disorganised that the heart
beats become weak and irregular, or in certain forms of heart failure in sinus rhythm.

Through the efforts of Stöck and Withering, experimental medicine was now able to
exploit the single greatest breakthrough in the history of drug discovery when alkaloids were
first extracted from plants early in the nineteenth century. Progress from prehistoric times
until then had been pitiful because two essential factors were missing, namely access to
chemical compounds with consistent potency and effective methods of clinical investigation.
Access to these during the past two hundred years has made it possible to identify and exploit
beneficial drugs, free from the dogmatic teachings that were the legacy of the past and a major
obstacle in the path of drug discovery.

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The first significant challenge to the polypharmacy of Galen and ibn Sina came from a man whose writings seethed with alchemical mysticism. Theophrastus Philippus Aureolus Bombast von Hohenheim (1493–1541), who gave himself the name Paracelsus, undermined conventional medical practice after his controversial writings were published posthumously by his admirers. A similar attack on the anatomical principles espoused by Galen came from Andreas Vesalius of the University of Padua in 1543 with the publication of *De Humani Corporis Fabrica* (On the Structure of the Human Body).

Paracelsus was born in Einsiedeln, near Zurich, the son of a physician who moved to a mining area in the south of Austria. It was there that he acquired an extensive knowledge of minerals and alchemy that was to later influence his medical practice. Paracelsus attended classes at several European universities, but was unimpressed with his teachers and their theorising about Greek science and medicine. He came to the conclusion that there was more to learn outside the universities than in them and he may never have qualified in medicine. Taverns were, for him, ideal venues for acquiring local knowledge about what folk remedies might be effective. With his singularly uncouth manner, taverns would have been ideal for him to mix with people, especially during the ten years in which he travelled the length and breadth of Europe and into Arab lands in search of what he believed were the latent forces of nature.

By the time Paracelsus had returned from his travels, his fame had spread across Europe and as a result of successfully treating the influential publisher Johannes Frobenius, he was appointed as municipal physician and professor of medicine in Basle in 1527. In the face of strong opposition from the academic community, he was denied an official position in the university. Their objection can readily be understood, for he would neither produce his medical qualifications nor take the oath of Hippocrates. A few weeks later, he publicly burned copies of ibn Sina’s *Canon of Medicine* during St John’s Day celebrations. He attracted students from all over the continent to his lectures, but nearly a year later he had to flee the town after denouncing a magistrate who found against him over a disputed fee for treatment. During the remaining years of his life he travelled around Europe and wrote his *Der Grossen Wundartzney* (The Great Surgery Book), which was published in 1536.

With his knowledge of alchemy and the theory of transmutation, Paracelsus believed that there were three elements, mercury, sulfur and salt. These were not the materials with which we are familiar; rather they were characteristics of these, namely volatility, combustibility and cohesiveness, respectively. Though this was wrong, it encouraged Paracelsus and his followers to introduce chemical remedies that would never have been considered under the humoral system as they lacked the properties of being moist, dry, hot or cold. He also thought that within every object there was some sort of volatile spirit, the quintessence, which had counterparts both in the stars and in creations of the natural world such as plants and minerals. For him, this explained why certain herbs and mineral salts could influence specific types of disease. His upbringing led him to turn to alchemy to find the quintessences from herbs and minerals in their uncontaminated state, as he supposed they existed at the time of the creation of the universe. He believed that, with the passage of time, impurities had accrued,
but if these were removed then minerals could safely be given by mouth as medicines. This approach led Paracelsus to adopt monotherapy with purified compounds and was, in the long term, of the utmost importance in the development of drug discovery. Any therapeutic benefit could now be directly attributed to a specific drug, whereas with the polypharmacy of Galen and ibn Sina there was little likelihood of this. The ultimate acceptance of the benefits of cinchona bark and ipecacuanha roots in the century after his death was a direct result of their being administered on their own.

Much that Paracelsus wrote was quite absurd, for he would consider almost any report or claim with equanimity on the grounds that it should be put to the test before judgement could be passed on it. The outcome of this was that magic, superstition and the occult, together with obscure alchemical investigations and philosophical enquiries, often dominated his writings. Few of these were published during his lifetime, but many of his manuscripts were recovered some years after his death and then printed. Their obscurity and mysticism encouraged others to expand upon and develop his ideas with greater clarity, resulting in their wider acceptance. This set in motion a sequence of events that ultimately led to the isolation and identification of therapeutically effective chemicals.

THE PARACELSIANS

Konrad Gesner (1516–1565) was one of the first to follow in the footsteps of Paracelsus by favouring chemical medicines. A Swiss polymath who is better known as the father of bibliography on account of his famous Bibliotheca Universalis, he also wrote De Secretis Remediis, published in 1552 (translated into English under the title The Treasure of Euonymus), in which he recommended the use of chemical remedies. He also prescribed mercury, lead and antimony. Like Paracelsus, he tried to remove impurities from his compounds. One of his favoured methods for doing so involved distillation which ‘...corrected the malignitie or venimous qualities thereof, as in oyles of quicksilver, of oyle of vitrioll, antimonie...’. Crude forms of distillation had been used by the Greeks and were improved by Arab alchemists, but it was not until around the twelfth or thirteenth century that an efficient glass still-head was developed, enabling fractional distillation to be achieved. This allowed Arnald of Vilanova (c. 1240–1311), the physician who established Montpellier as the leading European medical school, to prepare a variety of distilled waters from plants. In the following century, the Spanish Franciscan monk Joannes de Rupescissa distilled from wine what he called aqua vitae, the water of life, believing it came from the heavens as the quinta essentia, the fifth essence, and as such was a panacea for all ills.1 This was the first recorded preparation of concentrated alcohol. Rupescissa’s text was circulated in manuscript until a printed version appeared in 1514, entitled Liber de Consideratione Quintae Essentiae. A few years before this, in 1500, the illustrated Liber de Arte Distillandi, de Simplicibus by Hieronymus Brunschwig (1450–1512) was published in Strasbourg. As a result of the appearance of these two books, distillation now became important in the manufacture of medicinal substances, and hence its use by Gesner.

The first of the so-called Paracelsians to express strong support for the views of Paracelsus was Petrus Severinus (1542–1602), physician to the King of Denmark. His Idea Medicinae Philosophicae was published in Basle in 1571. It exposed the inadequacies of the Galenic approach, described what was later to become known as the doctrine of signatures and urged its readers to burn their textbooks, then experiment and travel so as to gain first-hand knowledge of the natural world. Oswald Croll (c.1560–1609), who studied orthodox medicine at Heidelberg, Strasbourg and Geneva, became another early Paracelsian. His Basilica Chymica, published in Frankfurt in the year of his death, ran to many editions and included formulations still to be found in pharmacopoeias during the nineteenth century. His clarity of writing was in marked contrast to Paracelsus and the many editions of the Basilica ensured
that any physician or apothecary wishing to prepare a mineral medicine would find detailed instructions.

The views of the Paracelsians were disputed for many years, but refined mineral preparations were included in the *Pharmacopoeia Londinensis*, published by the London College of Physicians in 1618. As this was the most influential pharmacopoeia yet to have appeared, this was confirmation that these chemical remedies had finally become accepted in orthodox medical circles. From then on, their place in medicine was assured.

The earliest chemical remedies consisted of naturally occurring metals and their salts. As with plant- and animal-derived medicines, it is impossible to ascertain when they were first used as medicines, but some are referred to in ancient literature. Copper, gold, iron, lead, mercury, silver and tin were all known by Roman times. The writings of al-Zahrawi, especially the 28th volume of his *al-Tasrif*, provide detailed information on the preparation and purification of a range of mineral drugs. The range widened thereafter as alchemists conducted chemical reactions in their futile quest to convert base metals into gold. As a result of the rapid development of organic chemistry in the last third of the nineteenth century due to the growth of the dye industry, it became possible to manufacture organometallic compounds. Some of these were introduced into medicine, principally as chemotherapeutic drugs for treatment of infectious diseases. As such, they sometimes proved to be safer and more effective than their inorganic predecessors.

**IRON**

Iron appears to be the first mineral known to have been administered by mouth for therapeutic purposes. One of the oldest Greek legends tells how Melampus, a shepherd endowed with magical powers, cured the infertility of Iphiclus, King of Phylacea, by giving him wine in which rust scrapings from a knife had been steeped. Ferric oxide occurs naturally as a mineral, red haematite, and in the form of rust. Throughout recorded history, the astringent properties of ferric oxide have been exploited in various ways, such as in preparations for soothing the eye. It is mentioned in the *Ebers Papyrus* as an ingredient of a paste for the treatment of eye disease, while haematite in ox fat was applied to the eye by Babylonian physicians to alleviate photobia. The Hippocratic writings described the use of iron salts to stem bleeding, possibly resulting from the belief that battle wounds were best healed by application of the agent that caused them. Dioscorides noted that rust can stem uterine haemorrhage and also prevent conception, while evidence of iron therapy for debility during Roman times has been obtained from excavations in Gloucestershire, England. A detailed account of the use of iron by the Greeks and Romans was provided by Nicolas Monardes in the 2nd volume of his *Historia Medicinal de las Cosas que se Traen de Nuestras Indias Occidentales*. . . , where he cited them for conditions such as the bloody flux (amoebic dysentery), the flux of women (menstruation) and piles.

Lazarus Riverius, a physician to Louis XIV, in 1640 published the 15th volume of his *Praxis Medica*, a work that was translated into English 15 years later by Culpepper, Cole and Rowland as *The Practice of Physic*. This recommended steel dissolved in wine for chlorosis, a disease of young women sometimes described as love sickness, but now recognised as iron deficiency anaemia. The selection of an acid wine would have facilitated the oxidation of the iron. In 1681, Thomas Sydenham specified iron as the cure for chlorosis. He considered this to be a manifestation of hysteria caused by a weakness of the animal spirits, which could be cured by strengthening the blood as this was the origin of these spirits. He advised that the patient should be bled before starting a 30 day course of pills containing steel filings and extract of wormwood, each dose being followed by a draught of wormwood wine.

The Parisian apothecary Pierre Pomet (1658–1699) wrote his *Histoire Generale des Drogues*, which was translated into English in 1712 as the *Compleat History of Drugs*, with additions by
the Apothecary at the Jardin du Roi in Paris, Nicolas Lémery, and the medical botanist, Joseph Pitton de Tournefort. This recommended crocus martis, which is ferric oxide, for chlorosis and dropsy. A year after the publication of the English edition, Lémery and Geoffy provided a rational basis for iron therapy by demonstrating the presence of iron in ash prepared from blood.6

The first use of ferrous sulfate was by the French physician Pierre Blaud (1774–1858).7 This reduced form of iron occurs naturally as a hydrate in several minerals, but can readily be prepared by dissolving iron in sulfuric acid. In 1832, Blaud reported that he had cured 30 cases of chlorosis within 10 to 30 days by administering pills containing 320 mg each of ferrous sulfate and potassium carbonate. He believed earlier failures of iron therapy were due to the underdosing of patients. The amount of ferrous sulfate he administered was gradually increased until his patients were taking 12 pills daily, a dose that would now be considered excessive as 200 mg two or three times a day is recommended. Blaud’s nephew, a pharmacist, sold the ‘veritable pills of Doctor Blaud’ throughout the world.8 Many similar products appeared on the market and other ferrous salts were introduced, such as the carbonate, citrate, fumarate, gluconate, iodide, lactate, phosphate and succinate.

MERCURY

Since around 500 BC, the mines at Sisapo in Almadén (Arabic for the mine) in Southern Spain have been the principal source of Iberian cinnabar, red mercuric sulfide, which possesses a bright scarlet red colour that ensured its use throughout the ages as a pigment. Cinnabar was also the source of quicksilver, metallic mercury, which was of major importance to the alchemists. A scholarly account of the history of cinnabar and mercury by Goldwater has provided much of the information given here.9 He concludes that there is inadequate support for claims that either cinnabar or mercury were used medicinally in the ancient world by the Egyptians, Assyrians, Chinese or Indians. While mercury was prized above all other metals by the Indians, the date of its introduction into Indian medicine cannot be ascertained. Goldwater accepts that Hippocrates could have made use of cinnabar, but considers evidence of this is lacking.

Cinnabar was mined by the Etruscans until banned by the Roman Senate, possibly late in the second century BC, due to the damage caused to vineyards, olive groves and streams.10 Pliny the Elder (AD 23–79), who gave a description of how mercury was obtained by roasting cinnabar, pointed out that the importation of the equivalent of 1000 kg of cinnabar from Spain each year had become so important to the Roman economy that the price had to be fixed by law.11 Several of the prescriptions given by Celsus in his De Medicina, which was written around AD 30, contain cinnabar. In the applications listed it was employed as a purgative, which indicates internal administration. Cinnabar was also incorporated in salves for treatment of the eye disease trachoma and of genital ulceration, which could have been a venereal infection. In the following century, however, Galen warned that cinnabar was so poisonous that he would not even sanction its external application.

The appearance of syphilis in Europe at the end of the fifteenth century saw desperate measures being taken to cure this new plague. The old medicines failed miserably and healers and afflicted alike were prepared to turn in any direction to find a cure. It was against this background of near panic that mineral medicines such as mercury salves became established as a standard treatment for syphilis. The first book about syphilis, variously known as Consillium in Morbum Gallicum, Consillium in Pustulas Malas or De Morbo Gallico, was written by Conrad Schellig, a professor at Heidelberg, perhaps as early as 1488 and certainly no later than 1496.9 The last page of his book warned that great caution must be observed when administering mercury by inunction. At least eight others before the year 1500 recommended the use of mercury in the treatment of syphilis, but its strongest advocate was Paracelsus. He wrote 11 books on syphilis in which he rejected the use of guaiac in favour of mercury.
The term ‘syphilis’ first appeared in a revised version of a poem written in 1530 by the Italian physician Girolamo Fracastoro (1478–1553), in which the shepherd Syphilus is struck with the disease. The victim is advised to use mercury ointment: ‘Without hesitation, spread this mixture on your body and cover with it your entire skin... Ten days in succession renew this treatment... very soon, you will feel the ferment of the disease dissolve themselves in your mouth in a disgusting flow of saliva...’

This allusion to the flow of saliva reveals the rationale behind mercury inunction. Salivation was one of the many ways in which to expel harmful humours. This was clearly stated by the French surgeon, Ambroise Paré (1510–1590): ‘Verily we must so long use frictions and inunctions, until the virulent humours be perfectly evacuated, by spitting and salivation, by stools, urine, sweat or insensible perspiration.’

The influential seventeenth century London physician Thomas Sydenham (1624–1689) was similarly convinced that mercury cured syphilis through its ability to induce a profuse flow of saliva. This was still held to be true even in the middle of the nineteenth century, when Jonathan Pereira, another renowned London physician, described it as ‘...the great value of mercurials’. It was only after Fritz Schaudinn and Erich Hoffmann in 1905 isolated the spirochaete which caused syphilis, Treponema pallidum, that the capacity of mercury inunction to induce salivation was finally recognised to be nothing more than an indication of mercurial intoxication. It is, however, quite plausible that if mercurial treatment had an effect, it may have required a toxic dose to produce any benefit, hence the observation was made that salivation was necessary before a cure could be effected. As to whether mercury inunction ever did cure syphilis, it is the opinion of Goldwater that we shall never know since modern standards of clinical investigation cannot accept the reliability of the many records of patients who may have had syphilis and who may have been cured, especially since the spontaneous remission of the disease even without treatment was commonplace.

The influential Pharmacopoeia Augustana, published in Augsburg in 1564, included monographs on Unguentum Mercurium cum Theriaca, an ointment in which mercury was mixed with fat and other ingredients. However, in 1582 the Augsburg Senate included mercury and all its preparations in a list of substances that apothecaries were not permitted to dispense. Nevertheless, mercury later appeared in the pharmacopoeias of London in 1618, Brussels in 1671, Lille in 1694 and Valencia in 1698, reflecting its widespread acceptance in the treatment of syphilis by inunction. In the following century Thomas Dover acquired the name of ‘the quicksilver doctor’ after he published a monograph that hailed mercury as a panacea for all ailments. His lack of restraint produced an inevitable backlash, with controversy raging throughout the second half of the century. However, at the end of the century mercury was still being frequently prescribed, with Johann Friedrich Gmelin devoting an entire volume of his Apparatus Medicaminum to mercurial preparations. The nineteenth century saw even greater enthusiasm for mercury as it was prescribed not only for syphilis but also for testicular disorders, hepatic disease, plague, pleurisy, purpura, peritoneal inflammation, erysipelas, cholera, chronic diarrhoea, amenorrhoea, eczema, hydrophobia, chronic brain diseases, typhoid fever, amaurosis and inflammation. During the first half of the twentieth century alternatives became available to deal with all the established conditions for which mercury had previously been prescribed. Notwithstanding, ointments containing mercury appeared in all the major pharmacopoeias until the 1960s. The British Pharmaceutical Codex of 1959, for example, included strong mercury ointment containing 30% metallic mercury in a base of wool fat, white soft paraffin and beeswax. This kind of formulation had been the standard treatment for syphilis since mediaeval times, with patients smearing large areas of their body with it in the hope of a cure. Even after the introduction of the effective antisyphilitic drug arsphenamine in 1910, mercury ointments were widely used. That they remained in existence after the introduction of penicillin is disturbing.

In 1530, Paracelsus advised the city council of Nürnberg that mercury compounds should be taken in this way to treat syphilis. What became known as ‘blue pills’ were later widely used,
especially after their composition was revealed by the pirate Hairedin Barbarossa to François I of France, who then published it. These pills remained popular until the end of the nineteenth century, and the British Pharmaceutical Codex of 1954 still included mercury with chalk tablets as a purgative for children. It was omitted from the next edition as a consequence of the belated recognition of the harmful effects of mercurous chloride in infants.

Paracelsus recommended as a purgative a preparation in which mercury coagulated with egg albumin was mixed with alum water and distilled to form a powder which was actually red mercuric oxide. This oxide is less chemically reactive than the more finely divided yellow oxide, which is the one that has generally been used for medicinal purposes. A popular eye ointment known as Pagenstecher’s ointment was introduced in 1865, which contained 12.5% yellow mercuric oxide in spermaceti ointment. Variants on this appeared in most pharmacopoeias and were used to treat conjunctivitis, but they are no longer considered either safe or effective.

Mercurous chloride was discovered in the quest for a mercurial salt that would be less reactive than mercuric chloride (corrosive sublimate). Multhauf has suggested that this might represent the first product of deliberate chemical research. The earliest written account of its preparation appeared in 1608 by Joannes Renodaeus (Jean de Renou) in his Dispensatorium Medicum, who precipitated it from a solution of mercury in nitric acid by the addition of a solution of common salt. A year later, Oswald Croll in his Basilica Chymica recommended its administration by mouth to produce a catharsis that would expunge harmful humours from the body. It was included in the Pharmacopoeia Londinensis of 1618 as mercurius dulcis. Later, it became known as calomel, derived from kalos (Greek for beautiful) and melas (Greek for black) on account of the formation of a black powder on the addition of caustic alkali. The term is believed to have been introduced by the royal physician Sir Theodore Turquet de Mayerne (1573–1655), an enthusiastic advocate for the use of chemical remedies. Calomel is a white, tasteless powder that is practically insoluble in water. It was considered to be an ‘alterative’ for the relief of a diverse range of diseases and, like mercury itself, was among the most frequently prescribed drugs in the nineteenth century.

Many thousands were poisoned by calomel before infant teething powders containing it were withdrawn from the British market in 1954 after it was realised they caused pink disease, characterised by pinkish colouration of the palms and soles, fever, insomnia, lethargy, loss of appetite and pain in the extremities. The mortality rate was about one in ten. It was previously believed the symptoms were caused by teething, a complaint for which calomel was recommended. One such product was Steedman’s Powders, which contained 26.3% mercurous chloride. In 1953, Joseph Warkany, a Cincinnati physician collaborating with Donald Hubbard of the nearby Kettering Laboratory, produced convincing evidence that it was the mercury in teething powders that caused pink disease and it was promptly withdrawn from the market.

In the middle of the eighteenth century, Gerard Van Swieten (1700–1772) introduced the highly toxic mercuric chloride as a remedy for syphilis, employing a very dilute solution (0.1%) so that it could be taken by mouth. He had been curious as to whether the salivation produced by mercury treatment was a requisite part of the cure of syphilis. To settle the matter, he sought a mercurial preparation that could be diluted at will to allow administration of very small doses. After Robert Koch reported in 1881 that low concentrations of mercuric chloride were effective in destroying most of the micro-organisms that he had examined in vitro, Van Swieten’s solution and other similar formulations became popular as disinfectants. Their activity was due to the capability of the mercuric ions to form insoluble complexes with proteins.

A variety of other inorganic water-soluble salts of mercury were also introduced into medicine. Ointments containing mercuric nitrate were used until the 1960s to treat skin conditions, including eczema, psoriasis and syphilitic warts. Dilute solutions of mercuric cyanide were considered to be less irritating than those of mercuric chloride, although there may
well have been a parallel reduction in efficacy as a disinfectant. Another popular salt was red mercuric iodide, a naturally occurring mineral that can be rendered soluble by the addition of an equivalent weight of potassium iodide to form mercuric potassium iodide \( (\text{K}_2\text{HgI}_4) \). Gilbert’s syrup contained 0.05\% w/v mercuric iodide rendered soluble with potassium iodide for the treatment of syphilis. It remained in the *Pharmacopoiea François* until 1949. Ointments incorporating up to 2\% mercuric iodide were used in treating ringworm. Until the middle of the twentieth century, dilute solutions of mercuric oxy cyanide were used to bathe the eye for conjunctivitis as they were considered to be less irritant than solutions of mercuric chloride.

The first organomercurials appeared in the late 1880s. Mercury benzoate, which was slightly soluble in water, was one of several such compounds that were introduced in an attempt to reduce the irritancy and toxicity of inorganic mercurials by obtaining a slow, sustained release of mercuric ions from the organic complex. This was one of a few approaches to drug design at that time, as typified by the introduction of drugs such as chloral hydrate and urethane, which had been designed to release chloroform and ethanol respectively. A number of injectable organomercurials were subsequently marketed, including merbaphen, a double salt of sodium mercurichlorophenyl oxy acetate with barbitone that was introduced in 1912 by F. Bayer & Company for the treatment of syphilis. Seven years later a novel use was to be found for it after Arthur Vogl, a third year medical student at the Wenckebach Clinic in Vienna’s First Medical University, had ordered a 10\% solution of mercury salicylate to be prepared by the hospital pharmacy. When told that such a solution could only be prepared as an oily injection, Vogl accepted a colleague’s offer of merbaphen injection and duly proceeded with the treatment. As the nursing staff were meticulous in maintaining records of their patients’ progress, the amount of urine voided by every patient was routinely entered on a chart. Vogl saw at once that after receiving her first injection of merbaphen, his patient’s 24 hour urine output increased to 1200 ml from a mere 200–500 ml noted previously. After the third daily injection, this increased to 2 litres! On interruption of the treatment for a few days, the urine outflow decreased, only to rise on resumption of the injections. Vogl then administered merbaphen to another syphilitic patient, in whom the disease had damaged the heart, causing advanced congestive heart failure. Conventional diuretics had been tried on this man without success. Vogl found that after receiving the injection, his patient passed a massive amount of almost colourless urine. The flow continued throughout the day and the night and by the next morning he had eliminated over 10 litres. A similar profound diuresis was produced in other patients when injected with merbaphen, but not when they were given other antisyphilitic mercurials which were found to lack the potent activity of merbaphen. A thorough clinical evaluation was then carried out by Paul Saxl. This transformed the treatment of the severe oedema of congestive heart failure. By removing so much fluid, the pressure on the heart was relieved, allowing it to recover normal function. None of the many substances previously used as diuretics had anything like comparable activity. Nevertheless, it was soon recognised that merbaphen injections posed a risk of severe kidney damage or even fatal colitis. It was quickly superseded by another antisyphilitic agent, mersalyl, which was administered on an intermittent schedule to minimise toxicity.

![Mersalyl](image)

The diuretic action of mercury was originally observed long before its rediscovery in the Wenckebach Clinic. It had been employed by Paracelsus in his treatment of dropsy, but it was
not until the late nineteenth century that mercury and mercurous chloride were introduced as oral diuretics. Tablets combining finely dispersed metallic mercury with digitalis (a cardiac stimulant) remained on the market until rendered obsolete by the introduction of the thiazide diuretics in the 1950s.

**Ethacrynic acid**

In the 1950s, a novel approach to the problem of designing a non-toxic, potent diuretic was taken by a team of researchers from Sharp & Dohme, under the direction of Karl Beyer. Recent developments in renal physiology had convinced him that the moment was opportune for a fresh attempt to design a safe, effective diuretic. By then, it was known that reabsorption of water from the glomerular filtrate inside the lumen of the kidney tubules depended largely on the efflux of sodium ions across the tubule wall. It was believed that mercurial diuretics probably interfered with this movement of ions by inhibiting dehydrogenase enzymes inside the cells of the tubules. The ability of mercury compounds to bind to dehydrogenase enzymes (presumed to involve reaction with thiol groups) was readily demonstrable. This convinced the Sharp & Dohme scientists that it would be worth trying to design mercury-free inhibitors of dehydrogenases in order to avoid the toxic effects of mersalyl. It proved to be a daunting task that took many years of intensive research. The turning point was the realization that both merbaphen and mersalyl possessed a phenoxyacetic acid moiety. When an unsaturated ketone was attached to the 4-position of the benzene ring, potent hydrogenase inhibitors were obtained. Analysis of the influence of additional substituents revealed that chlorine or methyl groups attached to the benzene ring further enhanced potency. Ultimately, ethacrynic acid emerged as a safe, orally active diuretic in 1962, five years after a separate group of Sharp & Dohme chemists had announced their discovery of the thiazide diuretics with similar properties.15

![Ethacrynic acid](image)

Ethacrynic acid can trace its medicinal chemical ancestry all the way back to the introduction of pure mercury in the sixteenth century. Using the terminology previously promoted by the present writer, mercury can be considered as one of the oldest of all drug prototypes.16 The majority of drug prototypes come from the animal and vegetable kingdoms; mercury belongs to a minority that derive from the mineral kingdom. The development of many more therapeutic drugs from a single prototype is a theme that appears repeatedly in this book.

**ARSENIC**

Arsenic trisulfide (Greek: *arsenikon* = potent), orpiment, is a yellow ore that has been used since ancient times as a pigment. Another pigment was arsenic disulfide, red orpiment or realgar, which was used to dye hair, but it was arsenic trioxide that was the first arsenic compound to become established for medicinal use. It was originally obtained 5000 years ago as a by-product from the smelting of copper. There is, however, no conclusive evidence of the medicinal use of arsenic trioxide before the eleventh century, when ibn Sina recommended it for cancer, both internally and topically.17 From then onwards, salves containing arsenic formed the basis of popular remedies for cancer. While Paracelsus believed it to be effective in the treatment of cancer as well as ulcers and wounds, he warned that it was too toxic for
anything other than external use. He also fused arsenic with saltpetre to form potassium arsenate, which was dissolved in alcoholic solution to form Liquor Arsenici Fixi, a preparation later recommended by Jan Baptista Van Helmont in his *Ortus Medicinae* published in 1648. Van Helmont warned that this should only be used for external application. A similar preparation was introduced in the following century by Thomas Fowler, a medical practitioner in Stafford who published his *Medical Report of the Effects of Arsenic in Cases of Agues, Remittant Fevers, and Periodical Headaches* in 1786. He had carried out an investigation into the value of arsenic in the treatment of malaria as a consequence of his favourable results with a medicine popular in Lincolnshire, known as the Tasteless Ague Drops. Throughout the nineteenth century, Fowler’s Solution was widely accepted as a less reliable alternative to quinine for malaria, as well as for skin diseases, chorea, dropsy, hydrophobia and glandular obstructions. In the middle of the century, exaggerated reports that the inhabitants of Upper Styria in the Austrian Tyrol kept themselves in good health by sprinkling their food with small amounts of arsenic compounds led to the widespread use of it by the Victorians as a tonic. Even in the 1940s, Fowler’s Solution was being prescribed as a tonic to treat pernicious anaemia. This was probably inspired by the heightened colouring of the cheeks arising from an increased fragility of the blood capillaries consequent upon chronic arsenic poisoning!

In 1865, Heinrich Lissauer of Breslau reported in the *Berliner Klinische Wochenschrift* that he had ameliorated the condition of a young woman with acute leukaemia. She had presented with enlarged spleen and liver, together with a large number of white cells in her blood. Her disease was clearly advanced. Acting on the recommendation of a colleague, Lissauer administered Fowler’s Solution as a tonic, hoping it would improve his patient’s appearance. Her general condition improved dramatically, with the result that she was discharged from hospital five months later, only to die soon after. Lissauer disappeared from the scene, but the use of Fowler’s Solution in leukaemia persisted until after the introduction of the first modern cytotoxic drugs in the 1940s. In 1931, its use in chronic myeloid leukaemia was described. Reports from China of its intravenous administration to achieve remissions in patients with acute promyelocytic leukaemia appeared in the 1990s and were followed up by clinical investigations in the United States. In 2001, FDA approval was granted for a proprietary formulation of Fowler’s Solution by injection in promyelocytic leukaemia.

**Arsphenamine**

In 1858 David Livingstone, the Scottish medical missionary who explored much of Central Africa, recommended Fowler’s Solution for the alleviation of the symptoms of sleeping sickness. This was to take on greater significance after David Bruce demonstrated in 1896 that trypanosomes were present in the blood of animals afflicted with nagana, a form of sleeping sickness, and could be temporarily eliminated by administering Fowler’s Solution. Trypanosomes were protozoa somewhat larger than red blood corpuscles. Several varieties were subsequently shown to be responsible for what had previously been classified as 80 different tropical diseases affecting both men and animals. The most devastating of these in humans was sleeping sickness, caused by *Trypanosoma brucei* infection carried by blood-sucking tse-tse flies. In one recorded instance, the mortality had risen from 13 to 73% between the years 1896 and 1900. There were even fears that the disease could depopulate the whole of Central Africa, where it was being transmitted by the tse-tse fly, especially after an epidemic in Uganda killed 250,000 people in the first two decades of the twentieth century. Attempts to prepare vaccines proved futile.

In 1899, Alfred Lingard at Muktesar in India tried unsuccessfully to cure horses infected with the form of trypanosomiasis known as ‘surra’ by administering Fowler’s Solution. Three years later, Alphonse Laveran and Felix Mesnil at the Pasteur Institute announced that they
had been able to infect laboratory mice and rats with two varieties of the causative organism of trypanosomiasis. Subcutaneous injections of the sodium salt of arsenious acid into the infected rodents resulted in a rapid disappearance of trypanosomes from their blood, but the parasites reappeared within a few days to cause the death of the animals.26

Antoine Béchamp synthesised the sodium salt of the meta-anilide of phenylarsonic acid in 1863. At the beginning of the twentieth century it was marketed by the Vereinigte Chemische Werke in Charlottenburg with the unsubstantiated claim that it was 40–50 times less toxic than any of the inorganic arsenicals previously used. On this basis it was given the overly optimistic name of Atoxyl®.28 The director of the factory at Charlottenburg, Ludwig Darmstädtär, had been particularly interested in finding a cure for cancer and had sponsored some of Paul Ehrlich’s work in this field at the Institute for Experimental Therapy in Frankfurt. Whether Darmstädtär sent Ehrlich a sample of Atoxyl® with a view to its being evaluated in cancer or in trypanosomiasis is not clear. Atoxyl® was, however, the first substance Ehrlich investigated after receiving cultures of trypanosomes from Edmond Nocard of the Pasteur Institute. Unfortunately, it proved to be inactive.29 After this, Ehrlich began his investigation of chemotherapeutic dyes, which preoccupied him until 1905, when he was surprised to read a paper by Wolferstan Thomas, of the Liverpool School of Hygiene and Tropical Medicine, describing the success of Atoxyl® in the treatment of animals experimentally infected with trypanosomiasis.30 Ehrlich at once reopened his investigation of Atoxyl® and confirmed the validity of the results obtained by Thomas. He then realised that his earlier study had been misleading because it had been carried out on isolated cultures of trypanosomes, rather than on infected animals. This implied that Atoxyl® either stimulated immunity or else had to be metabolically converted to an active form by the animal before any trypanocidal effect could be exerted. Meantime, the bacteriologist Robert Koch was asked by the German Sleeping Sickness Commission to evaluate Atoxyl® in East Africa, where the mortality from the disease was reaching alarming proportions. Koch established that a 500 mg injection of the drug could cause the disappearance of trypanosomes from the blood for up to eight hours. His recommendation was that effective treatment would require constant medication for six months, a protocol that would present the risk of blindness through damage to the optic nerve in up to 2% of patients. This convinced Ehrlich that it would be worth his while examining analogues of Atoxyl® to see whether the ratio of efficacy to toxicity could be improved. Thanks to the generosity of the widow of Frankfurt banker George Speyer, who had donated a million marks, and also to John D. Rockefeller, who supported Ehrlich’s research, a chemotherapy institute was built alongside the existing Institute for Experimental Therapy. Called the Georg Speyer-Haus, it was officially opened in September 1906. There were excellent facilities for chemists to synthesise the analogues of Atoxyl® required by Ehrlich.

Ehrlich was fond of coining Latin aphorisms to express the complex ideas underlying his researches. To describe the principle guiding the design of the new chemotherapeutic agents he proposed to synthesise, he used the phrase corpora non agunt nisi fixata. This was his way of explaining that infecting organisms are not killed unless the chemotherapeutic agent has a high affinity for them. Ehrlich termed such drugs parasitotropic. Their toxic effects he attributed to their also being, to varying extents, organotrophic. His ideal agent would have a high therapeutic index because it exhibited a high parasitotropic activity and a low organotrophic activity. The therapeutic index could be measured simply by comparing both the curative and lethal doses of the test compounds in mice. Around the time he moved into the Georg Speyer-Haus, he began to accept the ideas on the existence of drug receptors newly propounded by the Cambridge physiologist John Langley. Ehrlich then advanced the hypothesis that the parasitotropic action of arsenicals was due to their binding to arsenoreceptors on the surface of the parasites, these being specific types of chemoceptors. It was his fervent hope that he could develop drugs that, like the antibodies discovered by his former colleague Emil von Behring, would act like ‘magic bullets’ insofar as they would bind only to receptors in the parasite and not the patient.
Initially, Ehrlich believed the scope for molecular modification would be limited since Atoxyl\textsuperscript{1} was known to be the chemically unreactive anilide of arsenic acid. His unexpected discovery that the drug reacted with nitrous acid to form a diazonium salt led him to the realisation that the structure originally assigned to it by Béchamp was incorrect. Ehrlich correctly concluded that Atoxyl\textsuperscript{1} was the sodium salt of 4-aminophenylarsonic acid, which meant that methods existed for preparing a wide range of its analogues.\textsuperscript{31}

In the spring of 1907, Ehrlich signed a formal contract with the nearby Cassella Dye Works, granting them exclusive rights to the commercial exploitation of his arsenic compounds in return for their sponsorship of his research. The arrangement continued after the Hoechst Dyeworks took control of Cassella in 1908. Ehrlich’s chief chemist, Alfred Bertheim, then synthesised a range of Atoxyl\textsuperscript{1} derivatives with substituents on the amino group. One of these was the N-acetyl derivative of Atoxyl\textsuperscript{1}, known as arsacetin. It was less toxic to mice, but when administered at the high dose level required to effect a cure it caused the mice to rotate in the manner of Japanese waltzing mice. This indicated damage to the vestibular nerve (associated with balance) similar to that produced by Atoxyl\textsuperscript{1}, and it suggested that arsacetin would likewise cause blindness. Recalling his earlier work on lead poisoning, Ehrlich became convinced that this nerve damage was due to chronic arsenic poisoning caused by the large amount of arsenic that had been administered. In seeking a more potent series of arsenicals he hoped to avoid this particular problem. To this end chloro, hydroxy, cyano, sulfonic acid and amino groups were introduced into the benzene ring, but all exhibited their own particular types of toxicity over and above neurotoxicity. None of them exhibited any enhancement of potency.

The discovery that higher concentrations of phenylarsonic acids were required to kill test tube cultures of trypanosomes than could possibly be achieved when curing infected mice led Ehrlich to conclude that they were undergoing metabolic activation, presumably through a process of chemical reduction. To test this hypothesis, he asked Bertheim to prepare the two possible types of reduction products from phenylarsonic acids. One type, the arsenoxides, were highly toxic to trypanosomes, but their general toxicity to the tissues of the host was also high. They were also very irritant when injected, due to the presence of impurities. The second series, the arsenobenzenes, were not as potent, although still more potent than the phenylarsonic acids, and they were less toxic to the host. This meant that small doses could be given to avoid neurotoxicity from chronic arsenic poisoning. Ehrlich subsequently restricted his investigations to arsenobenzenes, unaware that the neurotoxicity of his phenylarsonic acids was mainly due to impurities.

Ehrlich and his contemporaries believed that the arsenobenzenes consisted of two fragments linked by a double bond between their respective arsenic atoms. It was only years later that this was shown to be incorrect by Kraft and his colleagues in Russia,\textsuperscript{32} who established that the arsenobenzenes are polymeric mixtures formed from several molecules joined by single bonds between their arsenic atoms. Polymers cannot penetrate mammalian cells, which explains why the toxicity of arsenobenzenes was much weaker than that of the arsenoxides formed from only two molecules joined via arsenic–oxygen–arsenic linkages. The chemotherapeutic action of both arsenobenzenes and arsenoxides was shown in the 1920s to be due to
the release of arsenite molecules which had only a single arsenic atom that reacted with thiol groups in receptors on the surface of the parasites.

\[ \text{NaO}_2\text{CH}_2\text{CHN} \quad \text{NHCH}_2\text{CO}_2\text{Na} \]

Ehrlich’s structure for arsenophenylglycine

\[ \text{NHCH}_2\text{CO}_2\text{Na} \quad \text{NHCH}_2\text{CO}_2\text{Na} \quad \text{NHCH}_2\text{CO}_2\text{Na} \]

\[ \text{HO-As} \quad \text{As} \quad \text{As-OH} \]

revised structure for arsenophenylglycine

Arsenophenylglycine (compound 418) emerged as a promising trypanocidal agent. It was administered to patients in 1907 and for many it proved safe and effective. Unfortunately, in a small number severe and often fatal hypersensitivity to it was exhibited. Despite this, some physicians deemed it acceptable to continue to use it for those forms of trypanosomiasis with a high mortality rate. Ehrlich strongly disagreed.

Insertion of a hydroxyl group on the 4-position of the benzene ring was readily achieved by warming diazotised Atoxyl in water. Reduction of the product yielded arsenophenol. This was highly effective against trypanosomes, but was prone to oxidation to the severely irritant arsenoxide and exceedingly difficult to purify. However, Ehrlich’s prior experience with chemotherapeutic dyes (see below) convinced him that the introduction of a substituent adjacent to a phenolic hydroxyl group enhanced chemotherapeutic activity. An arsenophenol substituted in this manner was synthesised by Bertheim in 1907 as potential trypanocidal agent 606. The assistant who tested it told Ehrlich that it was ineffective, and consequently it remained on a shelf in the laboratory for over a year before being re-examined.

The isolation of the organism that caused syphilis, *Treponema pallidum*, was reported from the Reichsgesundheitsamt in Berlin by Fritz Schaudinn and Erich Hoffmann in 1905. The following year, Hoffmann visited the Georg Speyer-Haus and told Ehrlich that this spirochaete was in many ways similar to trypanosomes. He asked for samples of Ehrlich’s new compounds for testing on syphilitic patients in his clinic at Bonn, where he had just been appointed to the chair of dermatology. Ehrlich agreed, urging great care when using the arsenicals. Shortly after this, Uhlenhuth found that Atoxyl had a curative effect against syphilis in chickens, but was inferior to mercurials and could cause blindness through optic nerve damage. Ehrlich, meantime, arranged for his friend Albert Neisser, director of the dermatology clinic at Breslau, to take arsacetin and arsenophenylglycine to Java, where he had managed to inoculate apes with *T. pallidum*. The results were published in 1908 and confirmed the efficacy of arsenophenylglycine. This was the only way in which Ehrlich was then able to have his compounds tested on animals. The arrangement lasted until the spring of 1909, when Ehrlich was joined by Sacachiro Hata who had developed a method of infecting rabbits with syphilis while working in the Kitasato Institute in Tokyo. On his arrival, Hata was asked to test every arsenical that had been synthesised by Bertheim and his colleagues over the preceding three years. Working swiftly and with precision, he discovered that compound 606 had outstanding curative properties in the rabbits infected with syphilis! As a result, Ehrlich
arranged for the Farbwerke Hoechst to apply for a patent on ‘606’; the application was submitted on 10 June 1909. Further details of the synthesis were published later.35

Disappointment over the hypersensitivity reactions among patients receiving arsenophenylglycine led Ehrlich to exercise great caution over the assessment of ‘606’. Once the results of animal studies confirmed that it was likely to be safe and effective, samples of ‘606’ were sent to Iversen at the Obuchow Hospital for Men in St Petersburg and Alt at Uchtspringe (Altmark). Iversen was the first to administer the drug to patients when he successfully treated relapsing fever, a disease transmitted by lice. The causative organism, *Borrelia recurrentis*, was similar to the *T. pallidum*. Alt, on the other hand, spent three months evaluating the safety of ‘606’ in dogs before he would let two of his assistants volunteer to be injected with the new arsenical. Only after this was the drug administered to several syphilitic patients suffering from general paralysis of the insane. Once its relative safety had thus been confirmed, samples of ‘606’ were given to Schreiber at Magdeburg, where trials were initiated on large numbers of patients with primary syphilis. By January 1910, several other trusted investigators had received ‘606’ for trials.36 Ehrlich insisted on personally checking the records of every patient who received the drug, and it was not until the following April that he was prepared to make any public announcement about ‘606’.

On 19 April 1910, Ehrlich told the Congress for Internal Medicine at Weisbaden of his work leading up to the discovery of ‘606’. Hata then described his experiments and Schreiber explained how he had been able to cure syphilis with ‘606’. These announcements received enthusiastic applause from those present, and in the following days the press throughout the world carried headline reports of the new drug that could cure syphilis. The immediate result of this publicity was that the Georg Speyer-Haus was besieged by physicians and patients anxious to obtain samples of ‘606’. Letters also poured in from around the world. Under this unprecedented pressure, Ehrlich modified his policy of supplying the drug only to a small circle of trusted colleagues. He agreed to provide the physicians, at no charge, with a small number of vials of the drug that had been prepared under his supervision, on the condition that he received full reports of every case treated. Between April and December 1910, some 65,000 vials of ‘606’ were freely supplied in this manner while the necessary plant for its commercial production was being installed in the Farbwerke Hoechst. Production began there at the end of the year. ‘606’ was then marketed under the proprietary name of Salvarsan1, the approved name given to it later being arsphenamine. To the general public it remained known as either ‘606’ or ‘Ehrlich–Hata 606’.

Many syphilitic patients were cured by a single intravenous 900 mg dose of arsphenamine. This injection was prepared from powdered arsphenamine hydrochloride in a sealed ampoule. Prior to being injected, this had to be treated with the correct amount of dilute alkali to form a solution containing the highly unstable sodium salt, a procedure that brought many complaints from physicians. As more experience was gained, Ehrlich realised repeated dosing was necessary.38 Typically, 600 mg doses were repeated every few days until a total of 5 g of arsphenamine had been administered. Four to six weeks later, this was repeated and then
another one or two similar courses of treatment were required. In all, each patient would receive 25–30 injections.

What had happened in the Georg Speyer-Hause in the spring and summer of 1909 was the culmination of a truly remarkable effort. Ehrlich’s secretary, Martha Marquardt, has commented as follows:\(^39\)

“No outsider can ever realise the amount of work involved in these long hours of animal experiments, with treatments that had to be repeated and repeated for months on end. No one can grasp what meticulous care, what expenditure and amount of time were involved. To get some idea of it we must bear in mind that arsenophenylglycine had the number 418, Salvarsan\(^\text{R}\) the number 606. This means that these two substances were the 418th and 606th of the preparations which Ehrlich worked out. People often, when writing or speaking about Ehrlich’s work, refer to 606 as the 606th experiment that Ehrlich made. This is not correct, for 606 is the number of the substance with which, as with all the previous ones, very numerous animal experiments were made. The amount of detailed work which all this involved is beyond imagination.”

Despite early setbacks mainly because of careless administration, arsphenamine analogues remained the standard treatment for syphilis until the end of the Second World War, when adequate supplies of penicillin became available. There can be no question whatsoever that arsphenamine was the first major chemotherapeutic agent and that Ehrlich rightly deserves to be remembered as the founder of modern chemotherapy.

Shortly after starting to produce arsphenamine, the Hoechst Dyeworks patented neoarsphenamine (Neosalvarsan\(^\text{R}\)), a water-soluble derivative that was made less sensitive to oxidation by condensing some of the amino groups on the polymer with sodium sulfoxylate, an antioxidant widely used in the dyestuffs industry.\(^40\) Trials revealed this to be less potent than arsphenamine. This worried Ehrlich, but he was reluctantly persuaded that it should be made available because of the preference of the medical profession for a preparation that required only the addition of water to the ampoule immediately before injection. Neoarsphenamine ultimately superseded arsphenamine, usually being co-administered with either mercury ointment or oral mercury. It also became the standard treatment for anthrax until the introduction of antibiotics.\(^41\)

In 1919, Walter Jacobs and Michael Heidelberger at the Rockefeller Institute in New York introduced tryparsamide, an arsonic acid analogue of Atoxyl\(^\text{R}\).\(^43\) It had the valuable property of being able to penetrate the central nervous system and combat the central spread of the trypanosomiasis. Unfortunately, it also caused optic nerve damage in some patients, but until the early 1960s it was the drug of choice for cases of human trypanosomiasis. The problem of resistance led to its abandonment apart from when used in the treatment of Gambian sleeping sickness.
The Rockefeller Foundation patented tryparsamide, but they issued licenses free of charge to manufacturers who wished to produce it. It was truly fitting that the Rockefeller Institute, founded in 1901 with an endowment of $200,000, should succeed in fulfilling Ehrlich’s original objective only four years after his death. John D. Rockefeller had been one of the first backers of Ehrlich’s work on arsenicals.

Ernest Fourneau at the Pasteur Institute questioned Ehrlich’s belief that the neurotoxicity of phenylarsonic acids was due to the large doses required. Fourneau was convinced that the blindness caused by AtoxyI® was due to impurities, and this led him to examine thousands of phenylarsonic acids, from which acetarsol emerged as a valuable antisyphilitic and amoebicide. Acetarsol had been used in 1911 as an intermediate in the preparation of some of Ehrlich’s arsphenamine analogues. Fourneau arranged for it to be manufactured by Poulenc Frères under the proprietary name of Stovarsol®, a pun on his own name (French: fourneau = stove).

The product formed when solutions of arsphenamine were exposed to the air was found to be a highly potent antisyphilitic agent and possibly a safer drug than earlier arsenicals. It was introduced as oxophenarsine and was welcomed by physicians in the United States because it overcame the problems associated with the administration of earlier arsenicals with regard to solubility, stability and local irritancy on injection. The reason for this can now be appreciated, namely that oxophenarsine was a pure compound rather than a polymeric mixture. Another advantage was that it could be chemically assayed instead of having to undergo bioassay for standardisation. One indication of its superiority over earlier arsenicals is that it was still included even in the 1963 edition of the British Pharmacopoeia.
Working in his private laboratory (originally his kitchen!), Swiss chemist E.A.H. Friedheim attempted to find a trypanocide that would not damage the optic nerve. Concluding that the most potent of the existing drugs all contained nitrogen atoms, he introduced a melamine moiety because of its high nitrogen content, leading to the introduction of melarsen in 1938.\textsuperscript{46} It proved to be less toxic than existing drugs, but was much more expensive. Friedheim next prepared the trivalent analogue of melarsen, viz. melarsen oxide. This was more effective than melarsen, but also more toxic. Friedheim then tried to reduce its toxicity by reacting it with various thiols to mask the chemical reactivity of the trivalent arsenic moiety. Only after he received a supply of the arsenical poisoning antidote dimercaprol from the British Ministry of Supply was he able to prepare a safe analogue. This was melarsoprol (also known as Mel B), which was introduced in 1949 for the treatment of human African trypanosomiasis. It had a therapeutic index in mice infected with \textit{Trypanosoma equiperdum} 40 times greater than that found for melarsen oxide.\textsuperscript{47} Melarsoprol fulfilled its early promise and is still recommended by the World Health Organization for the treatment of human trypanosomiasis in combination with other drugs. Until recently, it was the only effective drug for advanced trypanosomiasis caused by \textit{T. brucei gambiense} or \textit{T. brucei rhodesiense}.

During the twentieth century, more than 12,500 arsenicals were synthesised in the quest for a safe trypanocide. Paracelsus would have been impressed. However, not all were made for the benefit of mankind. W. Lee Lewis of the US Chemical Warfare Service prepared a highly vesicant arsenical in 1918, known as Lewisite\textsuperscript{1}, which produced its destructive effects by penetrating the skin and then hydrolysing to form an arsenite.\textsuperscript{48}

Fears that Lewisite\textsuperscript{1} would be used by the enemy during the Second World War led the British Ministry of Supply to set up a team of scientists charged with the responsibility of finding an antidote. Based in the department of biochemistry at Oxford and led by Rudolf Peters, they established that arsenite reacted with two adjacent thiol groups on the vital pyruvate oxidase enzyme system. The discovery that this reaction could be competitively inhibited by the presence of simple sacrificial molecules that also contained two adjacent thiol groups ultimately resulted in the preparation of a highly effective antidote, namely dimercaprol (BAL, or British Anti-Lewisite).\textsuperscript{49,50} It was a viscous liquid that could be applied to the skin in an ointment, or else be injected. This wartime episode constitutes one of the finest examples of rational drug design, and dimercaprol is still used as an antidote to poisoning by arsenic, mercury and other metallic poisons that react with thiol groups in the pyruvate oxidase system.

\section*{ANTIMONY}

Red antimony trisulfide occurs naturally in stibnite, a mineral mentioned by Dioscorides and found in Hungary, France, Cornwall and Borneo. It appears in Arabic writings about eye diseases as well as in the \textit{Liber de Gradibus} compiled in the eleventh century by Constantinus Africanus,\textsuperscript{51} who introduced the term ‘antimony’. The medicinal use of stibnite was, however, limited by its insolubility in water. Paracelsus was largely responsible for the popularity of antimony trisulfide and other antimonial preparations from the sixteenth century onwards. He distilled the trisulfide from stibnite and recommended ointments and balms containing it for treatment of wounds, ulcers, leprosy and other skin ailments.\textsuperscript{52} Shortly before his death, the metallurgical text by Vannoccio Biringuccio, entitled \textit{De la Pirotechnia}, mentioned that a metal could also be formed from stibnite, although instructions for making this did not appear
until 1571 in *De Secretis Antimonii* by Alexander von Suchten. When finely divided, metallic antimony formed a slightly water-soluble oxide that could be taken by mouth as a purgative. It caused a number of deaths in Paris, provoking the Faculty of Paris in 1566 to persuade the Parlement to ban its use. The University of Heidelberg did likewise, but those who sought to evade these bans drank from cups of metallic antimony which, if filled with acidic white wine for 24 hours, could deliver a fatal dose of antimony tartrate.

Antimony and its compounds were hailed as a valuable remedy for syphilis, melancholy, chest pains, fevers and the plague in the *Triumphal Chariot of Antimony*, published in 1604 under the alias of Basil Valentine, supposedly a fourteenth century Benedictine monk but probably Johann Tholde (fl. 1595–1625). It had considerable influence, providing details of how to make a variety of antimony preparations. A gathering swell of opinion in favour of antimony ensued, which made the ban on it increasingly irrelevant. In the first half of the seventeenth century, Charles de Lorme grew rich and famous by prescribing antimony preparations for Henry IV, Louis XIII, Cardinal Mazarin and Madame de Sévigné. Eventually, the Parlement of Paris not only withdrew the ban in 1665, but voted by a large majority to recommend antimony potassium tartrate and other compounds as valuable medicines.

Antimony potassium tartrate was described by the German physician Adrian von Mynsicht in his *Medico-Chemical Treatise and Arsenal* of 1631. It became known as tartar emetic since a dose of 65 mg produced violent emesis and sweating. It was prescribed for fever on the basis that humoral imbalances were being corrected, reflecting the views of those who accepted chemical remedies without abandoning humoral medicine. It was used for this purpose until the introduction of modern antipyretic drugs in the late nineteenth century. A particularly successful product introduced in the mid-eighteenth century was known as ‘Dr James’ Fever Powders’, which combined the alleged virtues of antimony with those of mercury and remained on the market for over one hundred years. Another popular use of tartar emetic was as an external application when mixed with lard or in a plaster. This produced a burning sensation, followed by the appearance of a characteristic pustular eruption. It was thought to be beneficial in the treatment of severe ulcers. There was also a wider application, as can be seen from a letter written by Jenner (of vaccination fame) in 1822 which advocated the inunction of the shaven head with a tartar emetic ointment as a cure for insanity.

During the nineteenth century the popularity of antimonial drugs declined, but a reversal of this occurred at the start of the twentieth century after Alphonse Laveran demonstrated the ability of Fowler’s Solution (dilute arsenic trioxide) to kill trypanosomes in experimentally infected mice, indicating a possible role in the treatment of sleeping sickness. His colleagues at the Pasteur Institute, Mesnil and Brimont, went on to demonstrate the temporary disappearance of trypanosomes from the blood of experimentally infected mice. They proceeded to show that when cattle infected with trypanosomiasis were injected intravenously with antimony potassium tartrate, a cure could be achieved if large doses were given. Patrick Manson, the Scottish physician who demonstrated that some tropical diseases were transmitted by insects, then cured several patients with repeated injections of antimony potassium tartrate. There were severe side effects which were later attributed to the large quantity of saline administered with the intravenous injections. The need for prolonged treatment was confirmed when Jean Kérandel, a French physician in the Congo who had become infected, started to treat himself. The consensus that emerged was that antimony potassium tartrate was too toxic to be administered to patients for the prolonged schedule of injections that would be required. The subsequent development of safer, less irritant organic antimonial drugs also failed to provide anything to rival suramin or the organic arsenicals.

After detecting a hitherto unrecognised protozoon, *Leishmania braziliensis*, in patients afflicted with a skin disease known locally as *espundia*, a young Brazilian physician, Gaspar de Oliveira Vianna, tested antimony potassium tartrate. In 1912, he reported that the injections...
cured the disease. Antimony potassium tartrate remained the standard treatment for cutaneous leishmaniasis until superseded by sodium stibogluconate, another antimonial, but leishmaniasis remains one of the most widespread of all communicable diseases in the world.

A common form of it is kala-azar, a visceral disease that affects millions of children in Africa, Central and South America, China, India, the Middle East and parts of Russia. Without treatment the mortality rate is in the order of 90%. In 1915, di Cristina and Caronia successfully treated children in Sicily by injecting antimony potassium tartrate. The intravenous injections caused irritancy and, on occasion, sudden death, but they have led to a reduction of the mortality rate from kala-azar to around 10%, saving the lives of millions of children. Few other drugs have ever made such impact.

In 1915, James McDonagh found schistosomiasis (bilharzia) also responded to antimony potassium tartrate therapy. His findings were overlooked until three years later when John Christorpherson in the Civil Hospital at Khartoum noticed that tartar emetic acted against schistosomiasis in a patient being treated for leishmaniasis. McDonagh then published a paper in the *Lancet* shortly after that by Christorpherson which had been published in the same journal. Treatment with antimony potassium tartrate on a large scale began in Egypt in 1919 and antimonials became the standard therapy for schistosomiasis until the introduction of praziquantel. Schistosomiasis still affects the lives of 200 million people, mainly in Egypt. The parasite is transmitted by fresh-water snails.

Antimony potassium tartrate had major drawbacks, especially the need for prolonged treatment and its serious side effects. This drove forward research into the development of safer, less-irritant analogues by Hans Schmidt at the von Heyden Chemical Works in Radebeul, Germany, for testing at the University of Freiberg by Paul Uhlenhuth who had previously found sodium 4-aminobenzenestibonate, the antimony analogue of Atoxyl, to be active *in vitro* but chemically unstable. The first successful product of this collaboration between Uhlenhuth and Schmidt was stibenyl, the antimonial equivalent of Ehrlich’s arsacetin. Stibenyl was introduced into the clinic in 1915 as it appeared to be less toxic than antimony potassium tartrate. It removed trypanosomes from the blood of infected patients for several months, but relapses invariably occurred. However, it was successfully used in treating kala-azar in children until superseded by stibophen, which had been developed by Uhlenhuth and Schmidt because they believed that the severe irritancy of antimony potassium tartrate was due to breakdown of the complex to release antimony oxide. They had set about preparing stronger complexes, resulting in the marketing in 1924 of the potassium salt of stibophen. Stibophen was re-formulated five years later as the less irritant sodium salt. It was astutely marketed under the brand name of Fouadin to impress King Fouad of Egypt, a country where schistosomiasis was, and remains, a major health problem causing debility in millions of people. Schmidt also synthesised sodium stibogluconate, a pentavalent antimonial
in which gluconic acid replaced tartaric acid, which was evaluated in the IG Farben laboratories at Elberfeld in 1937. It is now considered to be the drug of choice in all types of leishmaniasis, being rivalled by only meglumine antimonate, introduced by Rône-Poulenc in 1946. This is a pentavalent antimonial in which tartaric acid has been replaced by an aminosugar derived from glucose. It is one of the most effective drugs currently available for the treatment of kala-azar and other forms of leishmaniasis.

BISMUTH

Until the seventeenth century, bismuth was confused with antimony, lead and especially tin. Claude Geoffrey the Younger, a French nobleman, demonstrated that it was a unique substance in 1753. Several salts were then introduced into medicine, their structures being uncertain as they vary with the method of preparation.

Bismuth subnitrate was introduced into medicine by Louis Odier, a Geneva physician (1748–1817). Administered for the relief of irritated mucous membranes and also as an antacid, it was thought not to be absorbed from the gut. Bismuth subcarbonate was introduced in 1857 and the subsalicylate followed in the first decade of the twentieth century. The latter decomposed in the stomach, forming salicylic acid and insoluble bismuth salts. Such salts are now known to form a protective barrier to attack by gastric acid on ulcers.

Tripotassium dicitratobismuthate is a colloidal mixture of components. The mixture is soluble in water, but precipitates below pH 5 to form bismuth oxide and bismuth citrate. When administered by mouth, it precipitates in the stomach to form a complex with glycoprotein that protects exposed ulcers from attack by gastric acid and pepsin. More recently, ranitidine bismuth citrate has been introduced for the treatment of ulcers, combining the effects of ranitidine with bismuth.

Bismuth salts were tested during the eighteenth century in patients with syphilis and gonorrhoea, and again in 1889 by Balzer. After they had been found to exert bactericidal activity against Spirochaeta gallinarum in chickens, Constantin Levaditi and Robert Sazerac at the Pasteur Institute then introduced parenteral bismuth therapy for the treatment of syphilis in the 1920s, but found this less potent than arsenicals and more toxic. The best results were obtained with lipophilic preparations formulated in oil, such as bismuth ethylcamphorate. Oily injections provided effective depot medication by giving a slow, prolonged release of bismuth from the site of injection. Such formulations were frequently prescribed in combination with arsenicals, until they were rendered obsolete by penicillin.

GOLD

Gold is mentioned as a medicine in both the Ebers Papyrus and the Hippocratic writings, but it was only after the mineral acids were introduced in the fourteenth century that it became possible to prepare potable (drinkable) gold, aurum potabile, for internal administration by using soluble gold trichloride. Despite the inability to be absorbed from the gut into the circulation, it remained in frequent use as a medicine well into the twentieth century! While Paracelsus believed in the therapeutic value of potable gold, recommending it for paralysis, fevers, palpitations and various acute diseases, it was Johann Glauber (1604–1668) who was responsible for its lasting popularity. His book, called De Auri Tinctura, sive Auro Potabili Vero, quid Sit et Quommodo Differat ab auro Potabili Falso et Sophistico; quomodo Spagyricæ Preparandum et Quomodo in Medicina Usurpandum, was published in Amsterdam in 1651. This recommended gold trichloride for the relief of melancholy, epilepsy, sterility and uterine disorders. Two centuries later, Jean André Chrestien at the University of Montpellier likewise recommended gold sodium chloride for syphilis and, in addition, tuberculosis. His views on the latter seemed vindicated when Robert Koch told an international congress in
Berlin in 1890 that gold–cyanide complexes were the most effective of all known antiseptics against the tuberculosis bacillus, *Mycobacterium tuberculosis*, being active *in vitro* at a dilution of one part in two million.83 Unfortunately, the complexes could not cure infected animals.

At the Bacteriology Institute of the Hoechst Dyeworks in 1913, Adolf Feldt confirmed that it was the gold component rather than the cyanide that had been responsible for the toxicity of potassium gold–cyanide to mycobacteria.84 This led to the introduction in 1917 of Krysolgan®, a complex of gold and the sodium salt of 4-amino-2-thiophenolcarbonic acid. A few years later Hoechst marketed the water-soluble sodium salt of aurothiobenzimidazole carbonic acid (Triphal®) for injection in the treatment of tuberculosis.85 This was followed by another product from the same company in 1924, namely gold sodium thiosulfate (Sanocrysin®), a compound originally prepared in 1845.86 It was found by Holger Molegaard in Copenhagen to have beneficial effects in cattle with tuberculosis87. Unfortunately, it was rather toxic in humans, but its use became firmly established until 1931, when a randomised, blind clinical trial using a matched control group was carried out in Detroit. It was the first such trial to be conducted in the United States and it revealed that in two groups of 12 patients the control group given injections of distilled water responded more favourably than those receiving the gold injections!88 This effectively put an end to the use of gold therapy for tuberculosis.

Most gold thiolates were foul smelling liquids that oxidised in air, but an exception was aurothioglucose (Solganal®), introduced by the Schering Corporation of Berlin. It was later shown to be hexameric in aqueous solution. Unlike other gold thiolates, it was formulated as a suspension in oil. The resultant slow release of the gold complex may account for its apparent reduced tendency to produce side effects when compared with other gold complexes. Landé in Germany administered it to 39 patients suffering from tuberculosis, bacterial endocarditis or a variety of complaints attributed to bacterial infection, but in most cases probably due to rheumatic fever.89 He believed that gold complexes had a general antiseptic effect in addition to any possible antituberculosis activity. The most notable outcome of his ill-defined clinical trial was that joint pain was relieved in some of his patients who happened to have arthritis. This serendipitous observation led Landé to recommend a trial of the drug for arthritis. Jacques Forestier in Paris acted on this recommendation and began to administer once-weekly intramuscular injections of gold-thiopropanol sodium sulfonate (Allocrysine®) to patients with rheumatoid arthritis. Six years later he published a detailed report revealing that 70–80% of patients gained some benefit from the treatment, despite a risk of serious toxicity.90 Forestier advised that a large-scale clinical trial of gold therapy for rheumatoid arthritis should be conducted, but the outbreak of war prevented this. It was not until 1945 that the results of a properly controlled, large-scale, clinical trial of gold therapy for arthritis were published. Fraser reported from the Western Infirmary in Glasgow that improvement occurred in 82% of 57 rheumatic patients given intramuscular injections of sodium aurothiomalate (Myochrysin®), as...
opposed to only 45% of 46 patients who were given dummy injections. Such placebo responses to blank injections is commonplace, making evaluation of many drugs difficult. In the Glasgow trial neither patients nor their attendants knew if the injection contained the drug or not, making it a ‘double-blind trial’. Only after the Glasgow trial was its value in therapeutics generally accepted. As a consequence of the pioneering work of Forestier and the subsequent clinical trials, gold therapy is now firmly established, with sodium aurothiomalate being the most frequently administered injectable drug.

The gold compounds that have been described have low lipophilicity and high molecular weights due to their polymeric nature; consequently they are not well absorbed from the gut. The lack of an orally active gold preparation imposed a major limitation on gold treatment for arthritis. Blaine Sutton, however, found that highly lipophilic alkylphosphine gold complexes exhibited anti-inflammatory activity in rats when orally administered. The most potent of these was triethylphosphine gold chloride, which was as potent as injected sodium aurothiomalate. Unfortunately, it was highly toxic in humans, whereas auranofin, an acetylated glucose derivative of it, proved to be safe. Although somewhat less effective than injected gold thiolates, it was better tolerated by most patients. About 25% of an oral dose was absorbed from the gut, a reflection not only of its lipophilicity but also of its monomeric nature. The other gold complexes used in the treatment of rheumatoid arthritis are polymers, and as such cannot be absorbed from the gut.

SILVER

Metallic silver has seen little use as a medicine, although it was mentioned in both the *Ebers Papyrus* and in the Hippocratic writings. Paracelsus recommended it for treating what he described as complaints of the cerebrum, spleen and liver. The Dutch physician Franciscus de le Bœ (1614–1672) applied silver nitrate (*crystallis lunae*) to wounds, sores and ulcers, but it was in the following century that the topical use of fused silver nitrate, *lapis infernalis*, became established for this. Throughout the nineteenth century silver nitrate was applied to fresh burns and to remove granulation tissue from burns. Solutions, ointments or application by a stick of fused silver nitrate were all in use at that time. In 1887, Emil von Behring established that silver ions had antibacterial properties and recommended a dilute solution for treatment of gonorrhoea by irrigation. Six or seven years before this, the Leipzig obstetrician Carl Credé had begun instilling 2% silver nitrate eye drops to prevent gonococcal infection in new-born infants. This became established practice until the introduction of antibiotics, as did the application of silver foil to wounds, a procedure recommended by the American surgeon William Halstead.

There was revived interest in the use of silver nitrate solution following the publication of a report in 1965 on its value in the treatment of extensive burns, by Carl Moyer and his colleagues at the Department of Surgery in the University of Washington. Meanwhile, Charles Fox at Columbia University College of Physicians and Surgeons had been testing scores of
silver compounds over a nine year period before concluding that an ointment containing the silver salt of sulfadiazine was superior to silver nitrate in the treatment of burns infected with *Pseudomonas aeruginosa*. He licensed the product to Marion Laboratories in 1969.

**ZINC SULFATE**

Zinc occurs in the form of various minerals and the metal may have been obtained in ancient times from naturally occurring carbonates or sulfides. The water-soluble sulfate was prepared by dissolving zinc in dilute sulfuric acid. It was administered in solution as an emetic by Franciscus de le Boe in the seventeenth century. Two hundred years later it was considered to be the best emetic available for dealing with poisoning by narcotics as its action was rapid. In recent years, the use of emetics to treat poisoning has declined due to lack of evidence of any real benefit.

Zinc oxide occurs in nature as the mineral zincite. As it is insoluble in water, it was initially only applied externally in ointments for its absorbent and dessicant properties. It is still used for this purpose. However, in 1771, it was reported that in Amsterdam the German astrologist and quack Johan Christoffel Ludeman (1683–1757) had been administering a secret panacea called *Luna fixata*. Hieronumus David Gaubus, Boerhaave’s successor as professor of chemistry at Leiden, witnessed Ludeman using it to achieve apparently miraculous cures of convulsions in children. Consequently, he also began to prescribe zinc oxide, but cautioned that further evaluation of the treatment was necessary. It was still being prescribed for epilepsy in the middle of the nineteenth century, but doses of 5 or 6 g per day were used instead of the 20–30 mg used by Glaubius. This ill-founded treatment for epilepsy eventually became obsolete once effective drugs were introduced.

The medicinal use of oral zinc sulfate to reduce tissue copper levels in patients with Wilson’s disease was reported by G. Schouwink in 1961 in a thesis for the University of Amsterdam. He stated that the zinc sulfate had induced excretion of copper in the stools, but his findings were never published. His work was later followed up in The Netherlands by Tjaard Hoogenraad and his associates. However, it was a serendipitous observation by George Brewer and his colleagues at the University of Michigan Medical School that ultimately led to zinc sulfate becoming licensed for this purpose. On treating sickle cell anaemia patients with zinc it was discovered that this caused copper deficiency. They then began to treat patients with Wilson’s disease with zinc sulfate, but it was not until 1997 that the FDA approved this.

In patients with the potentially lethal genetic disorder known as acrodermatitis enteropathica, zinc absorption is defective and zinc-containing enzymes are inadequately synthesised. Zinc sulfate tablets are successfully given to treat this condition.

**LITHIUM CARBONATE**

John Cade conducted an experiment in a psychiatric hospital in Melbourne in 1948 with the aim of obtaining evidence to support his conjectures as to the nature of manic-depressive illness. He had observed that patients afflicted with thyroid disorders sometimes behaved in a similar manner to those suffering from manic-depression. Extreme hyperactivity of the thyroid seemed to cause a form of mania, while a marked absence of thyroid function could be correlated with depression. The issue addressed was whether manic-depression could be due to either over- or underproduction of a hormone. Cade then collected urine from manic patients, schizophrenics, melancholics, as well as from normal individuals. To test whether an active substance was present, the urines were concentrated and then injected into guinea pigs. If sufficient urine was injected, the animals convulsed, fell unconscious and died. Cade noted, however, that the urines from manic patients were up to three times as toxic as any others. He
established that the toxicity was caused by urea, but as the amount of this in the manic urines did not differ much from that in normal urines it seemed possible that there might be something present that augmented the toxicity of urea. The first substance considered was uric acid, but Cade ran into difficulties when preparing solutions of this highly insoluble compound. He overcame this problem by using its most soluble salt, lithium urate. On injecting a saturated solution of this to the urea that had been added to test his hypothesis, Cade found the toxicity was unexpectedly low. A protective factor appeared to be present. Subsequent investigation confirmed that the lithium afforded protective action against the expected convulsions.

Cade next examined the effects of lithium carbonate injections. He found that after a 2 h delay, the guinea pigs became lethargic and unresponsive. Two hours or so later, they reverted to normal behaviour, unharmed by the injections. As lithium salts had been used during the nineteenth century to treat epilepsy, gout and cancer, Cade considered it safe to test both lithium citrate and carbonate on himself. Finding no harmful effects, he administered 1200 mg of the citrate thrice daily to a 51 year old patient who had been in a state of manic excitement for five years. After five days, there was an improvement in his condition, and by the time three weeks had elapsed he was well enough to be transferred to a convalescence ward. During his fourth month of continuous treatment, he was released from hospital with instructions to take 300 mg of lithium carbonate daily, this causing less nausea than the citrate. Such was the improvement that the patient returned to his former occupation. Unfortunately, he became negligent about taking his medication and after six weeks without treatment he was readmitted to hospital on account of his irritability. Cade achieved similar success with nine other cases, the most impressive results being with excited patients. Cade's pioneering work was not widely recognised until 20 years later after it was put on a firm clinical foundation by the Danish investigators Mogens Schou and Poul Christian Bastrup. Today, lithium is considered to be a valuable agent for the prophylaxis of manic-depressive illness. Since it is a rather toxic drug, careful monitoring of blood levels is required, especially as the amount of lithium ion that enters the circulation varies markedly between different brands containing the same nominal quantity of lithium carbonate.

**PLATINUM**

Platinous chloride (PtCl₂), which had been prepared by dissolving platinum in a mixture of nitric and hydrochloric acids, was tried in the treatment of secondary syphilis in the nineteenth century. However, it was well over a hundred years later before platinum compounds became established as therapeutic agents.

In 1964, Barnett Rosenberg and his colleagues in the biophysics department at Michigan State University began to examine the effects of an electric current on a suspension of bacteria. They detected no change in the growth rate, but did observe abnormal elongation of the *Escherichia coli* cells, suggestive of interference with cell division. After extensive investigations, they came to the surprising conclusion that this must have been due to an electrolysis product being formed in the culture medium. It was then established that the action of ammonium and chloride ions on the platinum electrode was responsible for the formation of platinum ions. When similar platinum salts with the *cis* configuration were added to bacterial cultures, they produced the same effects on the bacterial cells. Several platinum compounds were then tested against transplanted tumours and were found to inhibit growth. The structural requirements for this activity were confirmed to be either a neutral bisamine or a primary amine complex with a *cis* configuration. Clinical trials of a compound known as Peyronie's salt confirmed its activity against human tumours, with the outcome that this received the approved name of cisplatin and was licensed for use in 1978.
Cisplatin has become established in the treatment of malignant teratoma of the testis and ovarian cancer. Tumours of the bladder, head and neck also respond. Cisplatin acts as an alkylating agent, forming cross-links both between and within the strands of DNA in the nucleus of dividing cells. A major problem has been its toxic effects on the kidneys and also the nausea and vomiting experienced by patients. The former has been ameliorated by administering cisplatin by slow intravenous infusion over 24 hours and fully hydrating the patient before and during treatment.

Platinum complexes prepared at the Johnson Matthey Technology Centre in Reading, England, were evaluated by the Institute for Cancer Research in Sutton, England, and Bristol-Myers and the National Cancer Institute in the United States. The discovery by Michael Cleare and James Hoeschele in Reading that malonate–amine platinum complexes had reduced general toxicity led to a variety of these being examined for both their effects on the kidneys of small animals, as evidenced by measurement of blood urea nitrogen levels, and on the white cell counts. From this it was apparent that the lack of chemical reactivity of the malonate ester complexes reduced their toxicity to the kidneys, albeit while increasing their activity against white cells. A total of about 1000 platinum complexes of all types had been screened by 1978, when seven were selected for detailed pharmacological evaluation on rats and parallel studies on their action against transplanted human lung carcinomas in mice. From these studies, carboplatin emerged as the only compound that was non-toxic at effective antitumour dose levels. Clinical trials confirmed that carboplatin was not only less nephrotoxic than cisplatin but also less likely to cause nausea and vomiting. It is administered by infusion for the treatment of advanced ovarian cancer and small-cell carcinoma of the lung. Oxaliplatin was synthesised in Nagoya City University, Japan, and evaluated at the Roger Bellon Laboratories in Neuilly, France, and then by Debiopharm in Lausanne, Switzerland. It is used in colorectal cancer in combination with fluorouracil.

MINERAL SALTS

Magnesium sulfate was isolated from the medicinal waters at Epsom in the south of England by Nehemiah Grew at the end of the seventeenth century. A factory was subsequently established at Shooter’s Hill, London, ensuring a cheap price for the salts. By the time supplies had run out in the middle of the nineteenth century, Epsom salts had become established as the most widely used purgative in England. Magnesium carbonate, which also occurs naturally, was commercially introduced at the beginning of the eighteenth century as an antacid and purgative. After Joseph Black distinguished magnesium oxide from lime in 1755, it also became established as an antacid and purgative.

A rival product was developed by Johann Glauber who became ill in Vienna in 1624 and then recovered after drinking water from a local spring. Believing that this was due to a constituent in the spring water, he extracted a salt which he called sal mirabile. It consisted of hydrated sodium sulfate and was later called Glauber’s salt and became a popular saline purgative. A later product to enter the market was Seidlitz water (aqua sedlitziana), from a spring in Bohemia. In 1724, Professor Friedrich Hoffmann found it to have a similar composition to Epsom waters. The product was first marketed under the name of Seidlitz Powders by the London pharmacist Thomas Field Savory and did not even contain magnesium sulfate; nor was it prepared from Seidlitz waters. It consisted of two packets of
salts, one containing tartaric acid and the other a mixture of sodium bicarbonate and sodium potassium tartrate. Upon mixing the contents of both in water, effervescence occurred. It was recommended for the treatment of a diverse range of complaints.122

ORAL REHYDRATION THERAPY

Fundamental advances in the understanding of intestinal transport took place in the 1960s. One of these was the demonstration by Stanley Schultz and Ralph Zalusky at the US Air Force School of Aerospace Medicine in Texas that sodium ions were co-transported when sugars were absorbed from the small intestine into the circulation.123 This became the basis for the reintroduction of oral rehydration therapy (ORT) to replace body fluids lost by infants with diarrhoea. Harold Harrison had pioneered this treatment in Baltimore in 1945, but commercial products based on his work contained excess carbohydrate and caused too much sodium to be absorbed, with consequent complications.124,125 ORT then fell out of use until after various formulations were evaluated in the 1960s. In 1978, the World Health Organization adopted ORT as its principal strategy for preventing the deaths of more than 4.5 million children from diarrhoea every year. The treatment was typically provided in sachets containing sodium chloride (3.5 g), trisodium citrate (2.9 g), potassium chloride (1.5 g) and glucose (20 g). The contents of a sachet were dissolved in 1 litre of clean water. Variants of this formula that could be readily prepared in remote villages were also devised. As a consequence of the introduction of ORT by United Nations agencies such as UNICEF, the annual deaths are estimated to have fallen to around 1.5 million in 1999. No other form of drug therapy has ever made such a major impact.

CHLORINATED DISINFECTANTS

Claude Berthollet, the famous French physician who taught Napoleon chemistry, having noted that an aqueous solution of the recently discovered chlorine gas prevented the foul odours of decaying organic matter, in 1789 recommended this as a disinfectant solution.126 Later that year, a similar chlorine preparation, Eau de Javelle (named after the Paris suburb of Javelle), was marketed. In 1825, Antoine Labarraque, a Parisian apothecary, introduced the similar Eau de Labarraque for wound disinfection.127 Two years later, Thomas Alcock did likewise for the purification of drinking water in the United Kingdom.128 His solution was included in the London Pharmacopoeia of 1836 under the name Liquor Sodae Chlorinatae. By the middle of the century, solutions of both sodium and calcium hypochlorites were firmly established as disinfectants.129

One of the first attempts to control an outbreak of the frequently fatal childbirth fever was that by the Dublin obstetrician Robert Collins in 1829 – one year before Oliver Wendell Holmes, in America, asserted that this disease was contagious, being transmitted by midwives.130 Holmes eventually was successful in stemming puerperal fever by insisting on hand disinfection with chloride of lime solution. When he reported his conclusions to the Boston Society of Medical Improvement in 1843, he met considerable hostility from many of its members who felt his views to be insulting.131 Similar opposition was experienced four years later by Ignaz Semmelweis, an assistant at the Lying-in Hospital in Vienna, when he showed that the mortality from puerperal fever in the First Division maternity ward, where medical students were trained, was higher than that occurring in the Second Division ward, where nurses and midwives received instruction. He argued that this was due to the students infecting the patients with putrid particles adhering to their fingers after post-mortem examinations. By insisting that students wash their hands in chloride of lime solution, Semmelweis ultimately reduced the mortality rate in his wards from 12% to just over 1%. However, this did not satisfy the conservatism of some of his senior colleagues. They resented his interference and he
became embroiled in bitter arguments, with the result that he was not reappointed when his post in the hospital was due to be renewed. He then had to move to Budapest, where his classical study, *Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers*, was published in 1861.\(^\text{132}\) It included striking mortality statistics that should have convinced all but the most biased, yet it failed to win over some of his critics and he became insane through the aggravation this caused. In 1865, the very year that Lister first used carbolic acid to prevent wound sepsis, Semmelweis died from a septic wound on his finger.

Wider use of sodium hypochlorite solution as a wound disinfectant resulted from investigations by Henry Dakin, begun at the University of Leeds at the outset of the First World War.\(^\text{133}\) For application to infected, open wounds, he recommended a 0.5% solution prepared by mixing chlorinated lime with sodium carbonate and boric acid. When this was reported to be too irritating for wounds, he replaced the boric acid with sodium bicarbonate. This formulation became known as Dakin’s solution. Its effectiveness was never in doubt, but there were problems when it was applied to wounds and delicate tissues. Unless special precautions were taken, both free alkali and chlorine were present, resulting in tissue irritation. This problem was overcome after further work was done by Dakin. He was aware that hypochlorite solution reacted with free amino groups such as are found in proteins. On examining the products, known as chloramines, he found these to have antiseptic properties. Their instability precluded clinical use, but Dakin and Cohen were able to exploit certain aromatic chloramines previously prepared by Chattaway at St Bartholomew’s Hospital, London, as these formed stable water-soluble sodium salts.\(^\text{134}\) From the Berter Laboratory at the Rockefeller Institute in New York, Dakin reported that chloramine-T was a powerful antiseptic that possessed all the advantages of hypochlorite solutions, but with lower irritancy.\(^\text{135}\) It acted by slowly releasing hypochlorous acid over a prolonged period, this rendering it highly efficacious in wound disinfection.

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In 1917, Abbott Laboratories in Chicago marketed halazone, which Dakin had developed to meet the wartime demand for tablets to sterilise drinking water.

**IODINE COMPOUNDS**

Iodine occurs in seaweed as well as various minerals. It was isolated in 1811 by Bernard Courtois, a saltpetre manufacturer in Paris.\(^\text{136}\) He observed the violet fumes of iodine when treating seaweed (a substitute for willow which had become unavailable during the naval blockade during the Napoleonic Wars) with sulfuric acid while attempting to isolate nitrates for the manufacture of explosives. Joseph Gay-Lussac then proved that iodine was an element.\(^\text{137}\)

Iodine was introduced into medicine in 1820 in Geneva by Jean François Coindet for patients with goitre after he had found it to be the only ingredient likely to account for any beneficial action of burnt sea-sponge, an old remedy for goitre that had been used since the middle ages.\(^\text{138}\) Coindet treated over 150 patients, but toxicity due to overdosing became a problem which, coupled with the general ineffectiveness of the therapy in patients with long-standing goitre, led to its eventual abandonment. In 1846, his townsman, Prevost, claimed that a local dietary deficiency of iodine might be the cause of goitre and cretinism. The obvious way to settle this matter was to carry out an analysis of iodine in food and water, but suitable
analytical techniques did not become available until the middle of the century. Even then, these lacked refinement and the issue was not settled until after the First World War, when David Marine in the United States finally confirmed Prevost’s suspicions. This led to the introduction of iodine supplements for inhabitants of goitrogenic areas.

It was not until 1896, when Eugen Baumann at the University of Freiburg detected iodine in the thyroid gland, that its role in the body became evident. Prior to this, there was no clear view as to what limit there was to the effects it had on the body. It had been assumed that the alleviation of goitre was due to a general stimulant action on all the glands of the body, which explains how in the nineteenth century iodine came to be used both in glandular diseases and as an ‘alterative’ to treat a wide variety of other diseases. It was as frequently administered to treat tuberculosis as it was for goitre. To cater for all these uses, the French physician Jean Lugol introduced an aqueous solution of iodine in 1829, rendering the iodine sufficiently soluble by the addition of two parts of potassium iodide for each of iodine. He used this preparation (subsequently known as Lugol’s iodine) during his clinical studies of 1829–1832. It became universally accepted as a way of administering iodine by mouth as an alternative to tincture of iodine, which was an irritant and caused a stinging sensation. Iodoform, which had been synthesised by G.S. Srullas in 1822, was recommended as an acceptable form of iodine for internal administration in goitre by a Newcastle physician, Robert Glover, in 1847. Another palatable alternative, especially if large doses were to be administered, was developed in Glasgow by Buchanan, who combined iodine with starch in a preparation that was introduced into the pharmacopoeias under the name of iodised starch.

In 1839, Davies, a physician in Hertford, used an iodine solution to disinfect wounds, a purpose for which it was later employed in the American Civil War. Nevertheless, iodine disinfection failed to gain general acceptance until the 1870s, when the French bacteriologist Casimir Davaine confirmed that iodine solutions could kill a wide variety of microorganisms. In 1878, iodoform was introduced as a hospital disinfectant by Apollinaire Bouchardat, the chief pharmacist at the Hôtel-Dieu in Paris. The mild disinfectant action, as well as any toxicity after absorption from open wounds, was due to small amounts of iodine being released on contact with the tissues, but it is doubtful whether iodoform had any value in the treatment of wounds.

Detailed studies on the value of tincture of iodine as a skin disinfectant in surgery appeared from 1905 onwards. Although it was widely used until the 1930s, it was superseded by the iodophors, aromatic compounds that release iodine. These are less irritant, odourless and do not stain clothing to the same extent as iodine. Examples include iodol (1886), soziodol (1887), thioform (1894), sanoform (1896), clioquinol (1898) and many more introduced in the twentieth century.

Chiniofon was marketed by F. Bayer & Company in 1893 and was strongly promoted for a diversity of conditions. In 1921, it was recognised to have value in the treatment of intestinal amoebiasis. A similar drug, clioquinol, was introduced in 1899 by Ciba. It became popular with travellers for the prevention of diarrhoea, but there was little evidence of its efficacy. It was withdrawn during the early 1980s after repeated reports from Japan of toxicity to the optic nerve.
Povidone (polyvinylpyrrolidone) is a water-soluble polymer of widely varying chain length that was developed by IG Farbenindustrie just before the Second World War as a plasma volume expander for the emergency treatment of shock, both on account of its resemblance to bilirubin and its colloidal properties.\textsuperscript{151} It was superseded by dextrans produced by \textit{Leuconostoc mesenteroides}, as these do not accumulate in reticulo-endothelial tissue.\textsuperscript{152} Following reports that povidone bound and detoxified various drugs in a manner similar to that of plasma proteins, Shelanski and Shelanski at the Industrial Toxicological Laboratories in Philadelphia investigated its effect on toxic inorganic materials. In the course of preparing a formulation with tincture of iodine, they found that a unique complex was formed between iodine and povidone and that it was no longer necessary to add solvent or solubilisers in order to dissolve the iodine. \textit{In vitro} tests for antibacterial activity were done and it was noticed that the povidone-treated iodine was less toxic to animals than was tincture of iodine. Skin tests on volunteers and then tests involving application to mucous membranes were followed by clinical trials, all of which confirmed the superiority of the product over other iodine formulations.\textsuperscript{153} It was marketed in 1955 with the approved name of povidone-iodine, since when it has become the universally preferred iodine disinfectant. It was originally believed that the iodine covalently bound to the povidone, but spectroscopic studies have since revealed that the iodine binds through hydrogen bonding of the hydriodic acid ($\text{HI}_3$) to carbonyl oxygen atoms near the end of the polymer chain.\textsuperscript{154}

POTASSIUM BROMIDE

Bromine was discovered in sea-water in 1826 by Antoine-Jérôme Balard, a pharmacy professor in Montpellier.\textsuperscript{155} He used it to prepare potassium bromide. Once the chemical similarity between bromine and iodine had been recognised, physicians attempted to use it as an alternative to iodine in the treatment of a wide variety of diseases, including syphilis and goitre.\textsuperscript{156}

The highly corrosive nature of bromine water led physicians to experiment with the potassium salt, which they then prescribed in massive quantities. In London, Williams claimed success in a case of enlargement of the spleen by administering potassium bromide given by mouth. This led directly to its introduction into the \textit{British Pharmacopoeia} of 1836.\textsuperscript{157} On 11 May 1857, the obstetrician Charles Locock presided at a meeting of the Royal Medical and Chirurgical Society of London at which the topic for discussion was epilepsy.\textsuperscript{158} He expressed the view, prevalent at the time, that many cases of the disease were due to masturbation, particularly in young patients. He also considered one type of epilepsy to be associated with menstruation. Locock told the meeting that he had met with little success in treating this until he chanced to read an interesting report by a German who became impotent after taking nearly 2 g of potassium bromide daily for two weeks. On withdrawal of the bromide, his normal sexual function had returned. Locock then administered bromide to several young women suffering from hysteria. Large doses were given with good results, so he proceeded to treat a patient who had suffered from ‘hysterical epilepsy’ for more than nine years. The result was striking. The epilepsy ceased as long as medication was continued, and all but one of 15 subsequent cases were said by Locock to be cured! Despite Locock’s eminence, little attention was paid to his comments. He did not publish any further details, and it was not until 1868 that the use of bromide in epilepsy came to be accepted when Thomas Clouston conducted a clinical trial to establish the correct dosage.\textsuperscript{159} The trial lasted for several months and demonstrated that patients experienced a reduction in the number of fits while receiving bromide. It was the first drug to be of proven value, despite its obvious drawback of only providing benefit at almost incapacitating dose levels. Potassium bromide remained the standard drug used in the treatment of epilepsy until after the introduction of phenobarbitone. It remained in use as a sedative until the introduction of the benzodiazepines rendered it obsolete. In some countries, potassium bromide is still incorporated in non-prescription
sedatives and hypnotics. Needless to say, the efficacy of bromide in epilepsy had nothing whatsoever to do with its effect on libido or sexual function!

INORGANIC COMPOUNDS AS DRUG PROTOTYPES

Inorganic drugs account for only a small proportion of all those in the modern therapeutic armamentarium. Their historical importance in general should not be overlooked, but in particular they deserve recognition for being the earliest purified products that were introduced into therapeutic medicine. They were also the first drug prototypes and, as such, have provided a legacy from the past that cannot be matched by the countless number of animal and vegetable materials that littered medical practice for thousands of years.

As has been seen, the first synthetic drug capable of curing a major disease was an organoarsenical called arsphenamine. Another organometallic drug, antimony potassium tartrate, has saved millions of lives. These two were introduced early in the second decade of the twentieth century. Since then, there have been many advances in the field of organometallic chemistry, especially in recent years. It would not be unreasonable to expect further therapeutic developments in this area in the future.

REFERENCES


The publication of the first textbook on human anatomy, *De Humanis Corporis Fabrica* by Andreas Vesalius (1514–1564), was a landmark in the history of medicine. It set in motion a continuing investigation into the structure of the human body that was to have far-reaching consequences. As an increasing number of dissections of human cadavers took place, major findings began to alter the understanding of the body, such as when the English physician William Harvey (1578–1657) discovered the circulation of the blood, which he reported in 1628 in his *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (*Anatomical Essay on the Motion of the Heart and the Blood in Animals*). This book challenged the teachings of Galen by disproving the claim that the body consisted of three linked systems – the brain and nervous system, the venous system and the arterial system. Galen had believed that the brain was the source of an animal spirit that facilitated sensation and thinking, whereas the liver was the centre of the circulatory system, from whence blood formed from food was transported around the body by the veins to provide nourishment. Blood that was consumed in this process was believed to be replenished as more food from the intestines entered the liver via the portal vein. The heart was not recognised as a pump, being considered merely as part of Galen’s third system that provided energy by carrying blood and vital spirit round the body through pulsating arteries. Again, it was thought that blood was consumed in the process; there was no conception of it being recycled.¹

After the Italian anatomist Marcello Malpighi (1628–1694) had used the microscope in 1661 to reveal the capillary network linking arteries and veins, the claims of Harvey seemed incontrovertible. During the next 150 years or so there were several attempts to reconcile the new anatomical findings with the views of Galen by formulating alternatives to the classical humoral system, which still retained the concept of disease arising from a disturbance of the equilibrium of the body. It may seem strange that in an age when leading thinkers were rejecting authoritarian religious doctrines, many physicians still wished to cling to the teachings of Galen, but this was often driven by their need to make a diagnosis and then take any necessary corrective action. There were also those who viewed the body as nothing more than a complex machine that obeyed the laws of physics, but they were challenged by others who believed the body was controlled by the soul or the mind.

It might be thought that with the various investigations into the nature of the body there would have been an opportunity to introduce innovative treatments, yet the drugs preferred by their proponents were neither novel nor selected through objective clinical testing; they were simply chosen on the basis of uncontrolled self-experimentation or theorising.² Once again, the process of drug discovery was to gain little from theoretical speculation about the nature of the body, and it was not until after active principles were isolated from plants in the nineteenth century that any significant progress was made. That particular breakthrough was not driven by theory but rather by the practical necessity of finding a way to detect adulterated herbal products.

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SOLIDISM

The physiological text *L’Homme, et un Traité de la Formation du Foetus (Man, and a Treatise on the Formation of the Foetus)* by René Descartes was published in Paris in 1664. It was in this work that Descartes professed his belief that all bodies, including the human body, are basically machines that operate according to mechanical principles. Among those who accepted this view were influential medical teachers such as Hermann Boerhaave (1668–1738), Professor of Medicine at the University of Leiden, which was situated near where Descartes had written his opus. Boerhaave tried to reconcile the new anatomical observations with Hippocratic medicine, in particular wrestling with the nature of the solid and fluid parts of the body in his *Institutiones Medicæ*, published in 1708. This portrayed the solids as composed of fibres that could also form the membranes of the vessels through which the fluids could flow. Boerhaave’s system of medicine portrayed disease as arising from a disturbance of the fibres, a rationalisation that his many students who came to Leiden from all over the world admired as an explanation of the diverse collection of observations on health and disease that their master had compiled. Boerhaave and others who thought that disease arose out of a malfunction in the solid fibres of the body rather than through humoral imbalance collectively became known as solidists. In reality, they had not abandoned humoralism; they had merely modified it in keeping with the times. Friedrich Hoffmann (1660–1742), the Professor of Medicine at the newly established University of Halle, thought that disease arose from alteration of the flow of fluid through the vessels, heart or muscles, resulting in alteration of the ‘tonus’, or tension, of the fibres, thereby inducing spasm or atony. In his *Medicina Rationalis Systematica* (1728–1740), he laid emphasis on the control that the nervous system had on movement, which was so important in the maintenance of good health. He accounted for the rapidity of the movement of animal spirits from the brain through nerves to the voluntary muscles by arguing that as the body operated like a hydraulic machine such fluids are speedily pushed along the tubes or vessels between its chambers. In disease, there is a disruption of this flow. However, he made no clear distinction between the nature of the fibres from which the tubes of the nerves, muscles and blood vessels were formed. It was left to Albrecht von Haller (1708–1777), a Swiss pupil of Boerhaave who became Professor of Medicine at Göttingen, to conduct animal experiments that showed muscles contract when deliberately stimulated, whereas nerves transmit pain or sensation. Von Haller had performed these experiments during his investigation into the irritability of different tissues, i.e. their ability to contract upon stimulation. His views on irritability appeared in his *Primaæ Lineæ Physiologiæ* (*First Lines of Physiology*), published in 1747. They so strongly influenced William Cullen (1710–1790), co-founder of the Glasgow School of Medicine and subsequently Professor of Medicine at Edinburgh, that he similarly named his work *First Lines of the Practice of Physic*, published in 1777, in which he taught his students that disease arose when nerves did not receive sufficient stimulation to provide the tonus that would maintain the flow of fluids needed for the normal function of organs. For many years, this remained the leading text on the treatment of disease.

The opinions of solidists such as Hoffmann, Boerhaave, von Haller and Cullen influenced the selection and use of drugs in the eighteenth and nineteenth centuries. The belief that disease arose from altered tone of the fibres in the nerves or blood vessels in affected organs led to attempts to increase the tone by using tonics (a term introduced by Hoffmann) or to reduce it with depletive drugs. A major benefit that arose out of this was a return to single-drug therapy, though polypharmacy did not disappear.

Once the concept of irritability had gained hold, diagnosis of hyperirritability was often made by the detection of an increased pulse rate, as this was a measure of the heart rate. Depletive drugs were then prescribed in order to reduce this, principally agents such as emetics, purgatives, diuretics, diaphoretics, antispasmodics, sedatives or narcotics. Since fevers were judged to arise from hyperirritability, the use of such agents became widely accepted.
However, when disease was considered to arise from decreased tone of the heart, blood vessels or other organs, it was deemed necessary to prescribe a tonic. The problem with this approach was that it was as open to abuse as humoral theory had been by those who wished to justify the use of any favoured remedy. Coupled with the unreliability of assessing whether a patient required depletive or tonic therapy, opportunities to justify therapeutic intervention were not missed by those so inclined, resulting in the introduction of a vast range of tonics for the heart, liver, kidney, brain, hair, skin and other organs.

It was in the hands of John Brown (1735–1788), a fellow Scot and former student of Cullen, that solidist theory took its most extreme form. His Brunonian system was expounded in *Elementa Medicinae*, published in 1780, in which he argued that the unique characteristic of animal and human life was ‘excitability’, particularly of the nerves and muscles. Excitability was steadily maintained by bodily and environmental stimuli which he termed ‘exciting powers’. Disease occurred when there was either a surfeit or deficit of excitability, the former condition being described by Brown as ‘sthenia’ and the latter as ‘asthenia’. He instituted an 80 point scale for indicating the degree of excitability of patients, with the normal state being mid-scale. Treatment was based on restoring the level of excitability to mid-scale by administering remedies that served as ‘stimuli’. In asthenic patients, this required stimulant or sthenic remedies supposedly acting with more energy than is normally appropriate for maintenance of good health, notably animal-derived food, wine, spirits, musk, camphor, ether and opium, in order of increasing strength. For sthenic patients, it was deemed necessary to administer remedies acting with less energy than that required for maintenance of good health, described by Brown as ‘antisthenic’ or ‘debilitating’. In addition to bleeding, exposure to cold and dietary restriction, this included drugs that produced vomiting, purging, sweating and all the other effects previously associated with depletive drugs. Here was yet another medical system that contrived to explain disease in terms of an imbalance or disturbance of the status quo of the body and then restore the balance with agents believed to have opposing properties. Again there was no attempt to assess the efficacy of the selected agents in an objective manner.

The absurdity of the Brunonian system can be seen in its use of cinchona bark. Believing that intermittent fevers were preceded by a state of debility, Brown had concluded that those afflicted with it were asthenic. This meant that they should be given a sthenic, stimulant drug to effect a cure. Since cinchona bark frequently cured patients, this was then taken as proof that it was a sthenic drug! By extension of this argument, cinchona was prescribed for a diverse range of stimulants, including alcohol, a substance that in no small part contributed to the early death of John Brown.

The Brunonian system was modified around 1800 by the Italians Rasori and Borda. The two classes of drugs were now described as stimulants or hypersthenics, and contrastimulants or hyposthenics. This approach rejected the Brunonian concept that all drugs were stimulants and instead accepted that some had the ability to depress excitability. Once again arbitrary arguments abounded. For example, it was claimed that since wine was a stimulant, anything that relieved intoxication from it must be a contrastimulant. Purgatives were no longer considered to be stimulants since they allegedly induced depression. However, opium was still considered to be a stimulant. This variation on the Brunonian system cannot escape the criticism that was applied to both systems by Pereira in the middle of the nineteenth century, when he pointed out that nine-tenths of the drugs then known possessed neither stimulant nor depressant activity. They were merely ‘alterative’ in their action. Pereira clearly recognised
that the failure of these systems lay in their classifying drugs by their secondary effects rather than their primary action.\textsuperscript{3}

The various medical systems that were in vogue during the eighteenth century encouraged physicians to prescribe from a constantly increasing range of drugs made available by the expansion of international trade. This all played a part in provoking Samuel Hahnemann (1755–1843) of Leipzig to introduce his homeopathic system in 1796, the first systematic account of which was published 14 years later as his \textit{Organon der Rationellen Heilkunde}. Hahnemann prescribed infinitesimally small doses of drugs, the selection of which was based on the principle \textit{similia similibus curantur} (like cures like). This was the opposite of that elaborated by Galen 16 centuries earlier, namely \textit{contraria contrariis curantur}, symbolising Hahnemann’s rejection of Galen’s antipathy, the use of drugs held to possess effects opposite to those caused by the disease itself. Hahnemann also rejected what he designated as allopathy, namely the use of drugs with effects that differed from those produced by the disease.

Hahnemann developed his system from a misunderstanding of what happened to him after he had taken a large dose of cinchona bark. It had induced nausea, vomiting and coldness of the limbs, probably caused by severe gastric irritation, but Hahnemann believed it was identical to the malarial paroxysm he had experienced on an earlier occasion. This led him to the conclusion that cinchona relieved the intermittent fever of malaria because large doses produced the nausea, vomiting and coldness associated with the disease.

Hahnemann also diluted his preparations to such an extent that no active material was left! At each stage of dilution, the container was vigorously shaken to increase potency through the release of spiritual powers. The year after he published his \textit{Organon}, Avogadro postulated that equal volumes of gases, at equivalent temperatures and pressures, contain the same number of molecules, but the number of molecules in a mole (6.022 $10^{23}$) was not calculated until later in the century. Hahnemann therefore remained blissfully unaware that for his popular 12C dilutions (i.e. containing 1 part of drug in $10^{24}$ of water) there was less than a one-in-two chance that a single molecule of any drug would be present.

In favour of homeopathy, it can certainly be said that it delivered many patients from exposure to the drugs favoured by adherents of the Brunonian and similar medical systems. There is some substance to his allegation that medicine as commonly practised at that time merely sought to evacuate the contents of the stomach and sweep the intestines clear of the materials assumed to cause disease. The contrast between the Brunonian and homeopathic systems was highlighted by the use of one of the most popular drugs of the day, namely cinchona bark – the one system grossly overdosed and the other hopelessly underdosed patients.

PNEUMATIC CHEMISTRY

In 1786, shortly after his breakthrough in understanding the role of oxygen in respiration, Lavoisier was visited in Paris by Thomas Beddoes, a young doctor who was keen to learn of the latest developments in pneumatic chemistry. Following upon his appointment as Reader in Chemistry at Oxford two years later, Beddoes acquired the reputation of being the leading exponent of pneumatic medicine in England. He was an adherent of the Brunonian system and believed that oxygen was the principle of irritability.\textsuperscript{5} He therefore administered air enriched with oxygen to his asthenic patients, while his sthenic patients were persuaded to inhale air deficient in oxygen. Beddoes continued with these experiments at Oxford until 1792, when his outspoken views on the merits of the French Revolution finally forced him to resign his post and establish an institution where patients could be treated with specially manufactured ‘factitious airs’.

Beddoes’ father-in-law and several acquaintances were members of the elite Lunar Society, quaintly named because it met monthly in Birmingham when the moon was full and so its
members could ride home safely. When Joseph Priestley had lived in Birmingham during the 1780s, he greatly influenced the philosophical activities of this small group. Other distinguished members included Josiah Wedgwood, the pottery magnate, William Withering, the physician who introduced digitalis into medicine, his rival Erasmus Darwin and the famous engineer James Watt, who had been persuaded by members of the Lunar Society to settle in Birmingham. It was to the Society, then, that Beddoes turned for patronage in 1793 after he published a pamphlet on the treatment of consumption (tuberculosis) by inhalation of ‘factitious airs’. Several members of the Lunar Society had good cause to support his efforts, for members of their own families were dying of consumption, including young Gregory Watt, the son of the engineer. Intrigued by the imaginative nature of Beddoes’ plans and their potential, the Society members backed him financially. Watt collaborated with Beddoes in designing a machine to manufacture factitious airs in 1794. Beddoes forged ahead with his plans to open what was to become known as the Pneumatic Institution for Relieving Diseases by Medical Airs. This opened in Clifton, Bristol, in 1798. In the basement was the massive machine built by Watt for the production of gases under the supervision of a young Humphry Davy, who was encouraged to experiment with new gases for patients to inhale. He examined nitrous oxide as an American, Samuel Mitchill, had claimed it was highly toxic. After establishing that small animals could be immersed in a jar of nitrous oxide without any apparent harm, Davy inhaled the gas himself, experiencing what he described as ‘...the most vivid sensation of pleasure accompanied by a rapid succession of highly excited ideas’. Later, he wrote in his laboratory notebook that, ‘Sensible pain is not perceived after the powerful action of nitrous oxide because it produces for the time a momentary condition of other parts of the nerve connected with pleasure’. Davy was a scientist, not a physician, and saw no therapeutic application in this. For Beddoes, the physician, his obsession with the Brunonian system convinced him only that Davy had found a stimulant for asthenic patients that did not have the drawbacks of alcohol. Davy never agreed with this interpretation. Neither of these historical figures recognised the significance of what had been found, reflecting the attitude of their time that pain was an inevitable aspect of surgery. Later, when chloroform anaesthesia was introduced into obstetric practice by Simpson, religious critics considered this deliberate removal of pain to be nothing less than a challenge by man against divine providence, quoting the words in Genesis III, ‘...in pain thou shalt bring forth children...’. Simpson ably brushed off this challenge by citing the creation of Eve in Genesis II, 21, ‘And the Lord God caused a deep sleep to fall upon the man, and he slept; and He took one of his ribs, and closed up the place with flesh instead thereof.’

\[ \text{N}_2\text{O} \]

Despite the neglect of its ability to numb pain, its euphoriant properties ensured that nitrous oxide soon became widely known, receiving the popular name of ‘laughing gas’. It was the most celebrated product of the Pneumatic Institution, which itself developed into a nursing home once physicians and patients alike came to realise that ‘factitious airs’ were not the panacea for all ills.

**SURGICAL ANAESTHESIA**

**Nitrous oxide**

Early in December 1844, Horace Wells, a dentist in Hertford, Connecticut, attended a public lecture at which Gardner Quincy Colton demonstrated the effects of laughing gas. Such lecture demonstrations of popular science were then in vogue, and men like the ex-medical student
Colton were able to earn a living touring towns around the United States. On this occasion, one of the audience who had volunteered to inhale the nitrous oxide accidentally gashed his leg while staggering around under the influence of the gas. Noticing that his injury did not seem to hurt the man, Wells pondered whether it might be possible to exploit nitrous oxide to ease the pain of dental extraction, a problem that he was particularly concerned with at that time. Two years earlier, he and his former partner in Boston, William Morton, had discovered the importance of the removal of entire decayed teeth rather than just the crowns. Although this was clearly in their patients’ best interest, such was the pain involved that few ever returned, and the dental partnership had to be dissolved.

After the lecture, Wells asked Colton if he would administer nitrous oxide to him while a troublesome molar tooth was removed. Colton duly appeared on the morning of 11 December 1844 and made history when he administered the first dental anaesthetic. Wells summed up the occasion succinctly, ‘A new era in tooth-pulling!’ Excited by his success, Wells went to Boston, where he contacted his former partner, Morton, and asked him to arrange a demonstration of anaesthesia in the presence of America’s leading surgeons in the Massachusetts General Hospital. Sadly, this was to prove his downfall. The demonstration was a miserable failure due to insufficient anaesthetic being administered. Wells was jeered out of the operating theatre in disgrace.10,11

Ether

The next substance to be investigated as a potential anaesthetic was ether, a volatile liquid which was synthesised in 1540 by Valerius Cordus through treating spirit of wine with oil of vitriol (sulfuric acid). Details of his synthesis of the oleum vitrioli dulce (sweet oil of vitriol) appeared in 1561 in De Artificiosis Extractionibus, a posthumous collection of his writings edited by Konrad Gesner that was later translated into English.12 Cordus thought ether had medicinal properties similar to those of sulfur, with greater penetrability because of its volatility. He recommended taking it by mouth to relieve pneumonia, pleurisy or a hacking cough. Sigismund August Frobenius, a German chemist working in Robert Boyle’s laboratory in London, described the preparation of ‘sulphuric aether’ to the Royal Society in 1730.13 Six years later, Friedrich Hoffmann described a mixture of ether dissolved in twice its volume of alcohol in his Observationum Physico-Chemicarum Selectiorum, published in 1736. This liquor anodynus minerali Hoffmanni became popularly known as Hoffmann's drops, being taken by mouth to relieve stomach, abdominal and pelvic pain. The preparation was often abused because of its alcohol content, which probably explains its continued popularity two hundred years later!

\[ \text{H}_3\text{C} \rightleftharpoons \text{O} \rightleftharpoons \text{CH}_3 \quad \text{ether} \]

A claim that inhalation of the vapour of ether produced much the same effects as nitrous oxide appeared in an anonymous article in the Quarterly Journal of Science and the Arts in 1818, which may have been written by Michael Faraday, an assistant to Humphry Davy, Director of the Royal Institution. As ether was obtainable from apothecaries, anyone who wished to experience its effects upon inhalation had little difficulty in obtaining supplies. This was to have far-reaching consequences in the 1840s when ‘ether frolics’ became popular in the United States and led to the discovery that ether inhalation could remove pain in much the same way as nitrous oxide.

Crawford Long, a young doctor in Jefferson, Georgia, was asked by friends to obtain nitrous oxide for inhalation at a party they were planning in December 1841. Long supplied ether instead of nitrous oxide and it proved a great success with the partygoers. So high-
spirited were the frolics that next morning Long and his friends found they had collected several bruises, none of them recollecting any pain during the party. Fortunately, Long had by now regained his wits sufficiently to realise the full significance of what had happened.14

There had, at that time, been much interest in medical circles in Mesmer’s claims that hypnosis could remove pain during surgery. Long had not been impressed by these claims, believing that mesmerism had only limited uses. Nevertheless, the idea of painless surgery now came back to him and he made up his mind to pursue the matter further if an opportunity arose. His chance came when, on 30 March 1842, he placed a towel soaked in ether over the face of James Venables, a young man who had frequently inhaled ether. As soon as his patient was insensible, Long proceeded to excise a large growth on his neck. The youth felt no pain. History had been made, but the country practitioner did not bother to report his success until 1849, by which time he considered he had acquired enough experience to tell the medical profession what could be achieved with ether anaesthesia.15

There was an even earlier use of ether a few weeks before Long anaesthetised James Venables, an event that was not reported until 1881 in Artificial Anesthesia and Anesthetics by Henry M. Lyman. In January 1842, William E. Clarke, a medical student in Rochester, New York, anaesthetised a woman undergoing a dental operation. Clarke also had experience of ether frolicks.16 This isolated case, like the experiments by Long, failed to influence the practice of surgery. However, the failed demonstration by Wells in Boston did have consequential effects through its influence on William Morton, his former partner in dental practice. Morton had remained dubious about the value of nitrous oxide for removing pain during surgery. He visited Wells and discussed its use before starting to experiment on his household pets in the summer of 1846.11 He mentioned his doubts about nitrous oxide to Charles Jackson, a chemist tutoring him for his medical studies. Jackson proposed ether as an alternative, a suggestion which was later to cause an unseemly row over who deserved the credit and financial rewards for the discovery of surgical anaesthesia. In retrospect, it has to be said that many chemists and physicians were at that time aware of ether as an alternative to nitrous oxide. Had not Long offered to provide it when his friends had been seeking laughing gas? Most of the credit must, therefore, go to Morton, whose endeavours were to ensure the general acceptance of ether anaesthesia.15

On 30 September 1846 Morton successfully maintained anaesthesia with ether for three-quarters of a minute while he extracted a tooth. For the next three weeks he used it on all his patients, observing that their exhaled breath contaminated the ether, which was held in a rubber bag. This resulted in a lack of uniform response. To avoid this, Morton designed an inhaler with a valve that vented exhaled breath into the atmosphere. The rubber bag was also replaced by a sponge-filled bottle, from which the patient inhaled ether through a tube between the lips while the nostrils were pinched shut. This new inhaler was ready just in time to be used in a public demonstration before a large gathering in the domed amphitheatre of the Massachusetts General Hospital on 16 October 1846. Morton administered the anaesthetic, whose nature he unsuccessfully tried to disguise by colouring it and labelling it ‘Letheon’. Gilbert Abbott, a 20 year old youth, was then presented to surgeon John Warren, who proceeded to make a deep incision several inches long near the lower jaw. Working with the swiftness and dexterity then required of a master surgeon, Warren removed a tumour without his patient feeling anything. The youth remained quite still most of the time. The following morning, the Boston newspapers carried reports of the operation. A month later, a paper on ether anaesthesia presented by Warren to the Boston Society of Medical Improvement appeared in the Boston Medical and Surgical Journal. No sooner did the world learn of the remarkable achievement in Boston than a row over priority of discovery of surgical anaesthesia broke out. Not only did Jackson challenge Morton’s claims, but so too did Wells, without whose investigations on nitrous oxide Morton would never have become involved in the quest to develop a safe anaesthetic.17 This dispute forced Morton to protect his own
interests by patenting ether, but this had little effect. Boston surgeons, however, followed up a suggestion in the patent by abandoning the use of the inhaler and instead anaesthetised their patients simply by pouring a few drops of ether at a time on to a sponge held over the mouth and nose. When London surgeon Robert Liston received reports of Morton’s demonstration, he had the eminent pharmacist Peter Squire fabricate an inhaler similar to Morton’s for use in an operation the following December. Subsequently English anaesthetists relied on inhalers, which they were forever improving to overcome a variety of design shortcomings. In Edinburgh, however, the influence of the American surgeons was stronger, with the open-mask technique prevailing. This continued to be used in Scotland, and on the Continent, well into the following century. As it was simple to use, there was little demand for specialist anaesthetists in Scotland when the more dangerous chloroform was introduced, whereas the English preference for inhalers necessitated specialist training.

**Chloroform**

Chloroform was first prepared in 1831 by three investigators working independently. Samuel Guthrie at Sacketts Harbor in the north of New York State prepared an alcoholic solution of chloroform by heating alcohol with chloride of lime in a copper still, naming the distillate chloric ether as he believed it to be the ethylene chloride (i.e. 1,2-dichloroethane) that he had been attempting to obtain.18 Around the same time, chloroform was prepared in France by Eugene Souberian and in Germany by Augustus von Liebig. The French chemist Jean-Baptiste André Dumas revealed the true nature of the dense liquid and both gave it the name of chloroform and elucidated its chemical structure.19

In 1842, the English physician Robert Glover demonstrated that dogs could be rendered unconscious by injections of chloroform, but he failed to recognise the significance this could have for surgery.20 It was the introduction of ether anaesthesia four years later that led to the discovery of chloroform as an anaesthetic. James Young Simpson, Professor of Midwifery at the University of Edinburgh, witnessed ether anaesthesia in January 1847, and became the first to use it routinely in obstetric practice. Two problems became apparent. Edinburgh was then the largest city in Scotland, with many of its inhabitants residing in high, crowded tenement blocks. Climbing up several flights of stairs on his domiciliary visits was difficult enough for Simpson, but having to carry the large bottles of ether required by the inefficient open-mask method was too much! This technique permitted wasteful evaporation of the ether from the mask into the atmosphere, and it was this that also caused Simpson’s second, more serious, problem. Gas lighting was by now installed in all the tenements; as Simpson proceeded to carry out his work, the concentration of ether in the air soon reached a hazardous level, posing the risk of a fire or even an explosion. Consequently, Simpson sought an alternative to ether. He was unaware that a solution of chloroform in alcohol had already been investigated at the Middlesex Hospital, only to be rejected on account of its cost. However, at St Bartholomew’s Hospital, also in London, William Lawrence found this so-called chloric ether to be a satisfactory alternative to ether. In Paris, Pierre Flourens, a former student of the physiologist François Magendie, had already tested both chloroform and ethyl chloride on animals. The latter was subsequently used in Ferdinand Heyfelder’s clinic in Erlangen, Germany, but it also was ultimately rejected because of its high cost.
Simpson began his search by testing a variety of volatile fluids on himself and his friends throughout the summer months. These fluids included Dutch liquid (1,2-dichloroethane), acetone, ethyl nitrate, benzene, and iodoform vapour. In October he met David Waldie, an old friend from the days when both had lived in Linlithgow. Waldie was now a pharmacist in Liverpool whose company supplied chloric ether to local physicians for inhalation by patients with respiratory disorders. This was made by dissolving chloroform in alcohol, so Waldie knew that chloroform was safe to inhale. Unfortunately, he was unable to supply Simpson with chloroform as his laboratory had just been destroyed by fire. Instead, Simpson acquired some from the Edinburgh druggists, Duncan, Flockhart & Company. In the company of a circle of friends gathered in the dining room of his home at 52 Queen Street on the evening of 4 November 1847, Simpson and two colleagues proceeded to inhale chloroform from glass tumblers and quickly fell asleep. A few minutes later, all three had recovered unharmed by the experience. His niece then tried it and others present also did likewise. Simpson at once recognised that chloroform met all his requirements, and duly reported to the Medical-Chirurgical Society of Edinburgh that it had many advantages over ether: it was more potent, requiring only a small volume of liquid; the onset of anaesthesia was quicker; it was also pleasant to inhale, and it was simple to administer by impregnating a cloth held over the mouth. Moreover, it was also non-inflammable. On 12 November he published his findings in a pamphlet that sold over 4000 copies within a few days. A paper appeared in the *Lancet* the following week.

When London surgeons used chloroform with inhalers they experienced difficulties not previously encountered with ether, and several deaths from cardiac irregularities were reported. The problem lay in the design of the inhalers, which tended to exclude oxygen-containing air. The first specialist anaesthetist, John Snow, carried out animal and clinical experiments which established the importance of controlling the proportion of anaesthetic in the mixture of air and anaesthetic being inhaled. To this end, he designed improved apparatus, and sought out a safer volatile liquid from the increasing range of organic solvents then becoming available. Snow determined both the boiling point and the concentration of solvent when air was saturated with it, using these physical properties to compare one substance with another prior to animal experimentation. He sought a solvent with the physical properties of chloroform combined with the clinical safety of ether, but his early death denied him of his goal, although not before he had achieved worldwide fame by anaesthetising Queen Victoria with chloroform when she gave birth to Prince Leopold in 1853.

Chloroform was not the ideal anaesthetic, nor was it entirely safe. Only 11 weeks after its first use by Simpson, a death occurred in Newcastle during an operation to remove an ingrown toenail. Many more deaths followed, although there were no problems for the majority of patients; during the decade 1870–1880, the *British Medical Journal* reported 120 deaths from chloroform anaesthesia. Official enquiries held throughout the world during the second half of the century led to no solutions. It was not until 1911 that an explanation was forthcoming after Goodman Levy at Guy’s Hospital in London found in cats that chloroform produced death by causing sudden cardiac failure in a manner not previously observed. He found three cats in which fibrillation had produced complete absence of regular cardiac pulsation and he went on to show that exactly the same effect was produced by injecting adrenaline into cats lightly anaesthetised with chloroform. The following year, the American Medical Association recommended that chloroform no longer be used as an anaesthetic, advice that was ignored by many anaesthetists. Chloroform anaesthesia only began to diminish in frequency after it was demonstrated in 1937 that chloroform sensitised the pacemaker (controller of the cardiac conduction system) to the action of both endogenous and injected adrenaline, shortening the refractory period between electrical impulse propagation and thereby causing ventricular fibrillation.
Nitrous oxide
It was not until 1862, after others had successfully demonstrated the value of ether and chloroform, that Colton again tried nitrous oxide for dental anaesthesia. On this occasion, he so impressed the dentist concerned, Dr Dunham, that he was asked for information on how to manufacture the gas. On returning to Connecticut a year later, he learned that Dunham had successfully administered nitrous oxide to more than 600 patients. Colton then persuaded him and a colleague to join him in partnership in New Haven. There, in a period of only 23 days, they painlessly extracted over 3000 teeth. Colton then moved to New York, where he built up a highly profitable dental practice. He administered nitrous oxide to thousands of patients without mishap. Word of this soon spread throughout the United States and Europe, and by 1868, nitrous oxide was regularly used in London hospitals. Not long after, it became available as a compressed gas in cylinders.27

Ethylene
Ethylene was the first new anaesthetic to be introduced in the twentieth century. The sequence of events leading up to this began when William Crocker and Lee Knight, at the botany laboratory in the University of Chicago, were asked by carnation growers who shipped their products into the city to find out why their flowers closed up when stored in greenhouses and the buds also failed to open. Heavy financial losses had been incurred through this in the 1908 season, so Crocker and Knight began work at once. They traced the cause to ethylene in the gas used to illuminate greenhouses, confirming that as little as 1 part of ethylene in 2 million parts of air caused already-open flowers to close! Further investigations revealed that ethylene damaged a large variety of plants.28

Concern that ethylene might also prove toxic to humans led Arno Luckhardt, a colleague of Crocker and Knight, to expose frogs, rats and a dog to the concentration of ethylene (4% v/v) present in illuminating gas. Although the gas rapidly anaesthetised the animals, no results were published until a full investigation into the possibility of using ethylene clinically was completed five years later in 1923.29 Luckhardt had by then established that ethylene induced anaesthesia very quickly and pleasantly, while recovery was also rapid and less eventful than with existing anaesthetics. Just days before Luckhardt was ready to publish his work, Easson Brown, an anaesthetist at the Toronto General Hospital, read a paper to the Academy of Medicine in Toronto describing animal experiments that indicated ethylene might be a promising anaesthetic.30 It was subsequently used until the early 1950s, when there was mounting concern about the risk of explosion with anaesthetics, especially those that had no major advantage over newer agents.

Cyclopropane
Work at Toronto on the clinical evaluation of ethylene was carried out both at the General Hospital and in the department of pharmacology where Velyien Henderson extended the investigations to include the chemically related gas, propylene. This was also a promising anaesthetic when freshly prepared, but after storage in pressurised steel tanks a toxic impurity was generated, causing patients to experience nausea and even cardiac sequelae. The matter was not pursued and propylene was no longer used. Several years later, however, after a chemist, George Lucas, joined the research team, Henderson asked if he could hazard a guess
as to the likely nature of the contaminant. When Lucas suggested it might be cyclopropane Henderson asked that some be synthesised and tested on animals. In November 1928, two kittens inside a bell jar were exposed to low concentrations of cyclopropane mixed with oxygen. To the amazement of all present the kittens quickly fell asleep, and on removal from the jar recovered with no hint of any toxic effects.\(^{31}\)

\[
\text{H}_2\text{C} = \text{CH}_2 \quad \text{cyclopropane}
\]

Extensive investigations began at once, with the result that a year later Brown safely anaesthetised Henderson, confirming the safety of cyclopropane in humans. These observations were reported to a meeting of the Canadian Medical Association, but the hospital authorities then announced that they would not permit patients to be anaesthetised with cyclopropane. Brown had no choice but to ask Ralph Waters at the University of Wisconsin to carry out the first clinical trials. These were completed by 1934, confirming the status of cyclopropane as a valuable and potent anaesthetic.\(^{32}\) It is still in use 50 years later. The toxic contaminant in propylene was eventually traced to the presence of hexenes.

**Vinyl ether (divinyl ether)**

In the twentieth century it was no longer necessary to rely on serendipity to discover new anaesthetics. Chemists and pharmacologists had now become adept at designing structurally related derivatives of existing drugs, as was the case when Chauncey Leake at the University of California Medical School realised that ethylene lacked some of the desirable properties of ether as an anaesthetic. Believing it would be reasonable to synthesise molecules containing both ether and ethylenic functions, he had the physical properties of five such compounds examined. It was found that only vinyl ether, a novel substance synthesised for Leake by Randolph Major at Merck & Company, had a boiling point and partition coefficient similar to those of ether.\(^{33}\) Predicting that vinyl ether would be an anaesthetic, Leake and Mei Yu Chen then administered it by inhalation to mice.\(^{34}\) Their experiment was a success, perhaps too much so, for when vinyl ether was introduced clinically in 1930, anaesthetists found its main drawback was a tendency for it to produce too deep a level of anaesthesia very rapidly.

\[
\text{H}_2\text{C} = \text{O} \quad \text{vinyl ether}
\]

Reports of liver damage following prolonged administration may possibly have been exaggerated, but they certainly undermined the reputation of vinyl ether. On the credit side, it did not irritate the respiratory tract to the extent that ether did and recovery was quicker than with ether. Although eclipsed by the post-war development of the fluorocarbons, vinyl ether was an important landmark in drug design.

**Halothane**

Early in 1930, Thomas Midgley was asked by the management of General Motors to develop a non-toxic, non-flammable, low boiling point refrigerant for the company’s Frigidaire division. Together with his colleague Albert Henne, he reasoned that the atoms on the right-hand side of the periodic table of chemical elements were the only ones that could permit sufficient volatility for a refrigerant, yet most of those were likely to prove toxic. The inert gases, on the other hand, were already known to be highly volatile. By a process of elimination, Midgley concluded that fluorine should be incorporated into hydrocarbons. He rejected the
assumption that such compounds would necessarily be toxic, believing that the stability of the carbon-fluorine bond would be enough to prevent release of either fluorine or hydrogen fluoride. Once the first compound, dichloromonofluoromethane, was prepared, a guinea pig was immediately exposed to it in a bell jar and experienced no ill effects. Thorough toxicological studies then confirmed that fluorocarbon refrigerants were non-toxic under normal conditions of use. The first compound, dichloromonofluoromethane, was prepared, a guinea pig was immediately exposed to it in a bell jar and experienced no ill effects. Thorough toxicological studies then confirmed that fluorocarbon refrigerants were non-toxic under normal conditions of use.35

After the Second World War ended, a wider range of compounds became available as a consequence of the wartime development of stable fluorocarbons as solvents for uranium hexafluoride during the separation of uranium isotopes by diffusion. A total of 46 of these were synthesised at Purdue University by Earl McBee and then sent for pharmacological evaluation to B.H. Robbins at Vanderbilt University, Tennessee. It was found that the more volatile compounds were convulsants, whereas those containing heavy atoms such as bromine had high boiling points and were apparently safer anaesthetics than ether or chloroform.36 The most promising compound then tested would today be recognised as the analogue of halothane (see below) in which the chlorine is replaced by a bromine atom.

Scientists at the laboratories of Ohio Medical Products followed up Robbins' suggestion that more fluoro-analogues should be examined. By this time there was considerable concern over the explosive nature of oxygen-anaesthetic mixtures, but later the British Ministry of Health reported that during the years 1947–1954 there had been a total of only around 40 explosions in the course of 7 million operations carried out in the United Kingdom. The risk of explosions caused considerable apprehension among operating theatre staff, so much so that it made good commercial sense for Ohio Medical Products to develop a non-explosive anaesthetic. This proved to be a difficult task for their chemists since the first non-inflammable fluorocarbon to be tested lacked anaesthetic activity, whereas the first promising anaesthetic (which was the 24th fluorocarbon to be synthesised) turned out to be flammable, albeit less so than ether. This was fluoroxyne. Fluoroxyne was considered by John Krantz at the University of Maryland to compare so favourably with ether that, after extensive animal testing, he proceeded to administer it to a volunteer in April 1953.37 It was released for general use three years later, but its flammability rendered it inferior to halothane, which was by then undergoing its first clinical trials.

James Ferguson, the director of the general chemicals division of ICI, was unaware of the developments with fluorocarbon anaesthesia in the United States when he was reviewing his company's range of fluorocarbons in 1950. Their total lack of chemical reactivity convinced him that they should be examined as anaesthetics. Responsibility for the project was given to Charles Suckling, who selected fluorocarbons that satisfied a range of critical requirements drawn up by himself and James Raventos, a pharmacologist from the medicinals section of the company's Dyestuffs Division at Manchester. Their prime objective was to attain low toxicity. The other priorities were that the anaesthetic must be non-explosive, have the right degree of volatility, induce anaesthesia both rapidly and uneventfully, not cause respiratory irritation and be highly potent. The exacting nature of these requirements was to pay handsome dividends because, unlike the American development of fluoroxyne, the product eventually introduced remained unchallenged for many years – even though it was only the sixth compound synthesised by Suckling. It was first prepared in January 1953 and came quickly to the fore by meeting all the physicochemical and animal criteria.38,39 After three years of exhaustive testing it was finally put on clinical trial at Crumpsall Hospital in Manchester. Patients were smoothly anaesthetised, their circulatory systems functioned normally and
recovery was free from nausea in most cases. The new fluorocarbon was given the approved
name of halothane. It quickly became the most widely used inhalational anaesthetic
throughout the world, a pre-eminence that remained unchallenged until the 1990s, when
concern deepened over the fact that patients who were again anaesthetised with it could suffer
life-threatening liver toxicity. This led to the introduction of similar fluorocarbon compounds,
such as desflurane, enflurane, isoflurane and sevoflurane. These do not produce the toxic
metabolite that is suspected of causing the liver toxicity seen with halothane.

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The greatest advance in the process of drug discovery was the isolation of pharmacologically active substances from plants in the early years of the nineteenth century. The first to be isolated was morphine and the work was carried out in Germany, but had this not come to the attention of the leading French chemist of the day, Joseph Gay-Lussac, it could have remained an obscure piece of scientific investigation. Instead, French chemists were able to exploit the discovery and lead the way in isolating more active principles. The seeds of the French dominance in this area had been planted some years earlier by Antoine Fourcroy (1755–1809). The son of an apothecary, he had been supported financially while he studied medicine by influential members of the Société Royale de Médecine. Such was his talent for chemistry that the Société permitted him to participate in its analysis of mineral waters for the government even before he graduated. He soon rejected the approach of evaporating the waters to dryness, and instead used specific chemical reagents to determine which minerals were present. Fourcroy went on to work with Lavoisier and then devoted much of his career to the application of chemistry to medicine. In 1792 he published an analysis of St Lucia and St Domingo barks, which had been recommended as substitutes for cinchona bark in the treatment of malaria. For many years his study was considered a classic of vegetable analysis. Significantly, it stimulated others to examine cinchona and opium, the two most important vegetable drugs then in common use, and which were often adulterated by unscrupulous dealers.

New institutions of higher learning were established in France following the decision of the Convention in 1793 to suppress academic and professional bodies that had enjoyed privilege under the monarchy. Responsibility for medicinal analysis passed to the Société de Pharmacie and the Ecole Supérieure de Pharmacie, which opened in Paris in 1803. The first director of the Ecole Supérieure was a protégé of Fourcroy, Nicolas Louis Vauquelin, the discoverer of chromium and beryllium. As the leading analytical chemist in Paris, he responded to growing concern in medical circles over the variable quality of plant products such as opium by encouraging his faculty members and others to isolate the active principles from plants so that reliable chemical assays could be established.

It is not merely that the appropriate institutions were in place that accounts for the ensuing breakthrough in the isolation of active principles. The methodology of solvent extraction had also been developed, albeit in a different field of chemistry, with its origins in 1747 when the German chemist Andreas Marggraf observed crystals forming after beetroot had been extracted with brandy. The crystals tasted very sweet and Marggraf was able to show they were identical to those obtained from sugar-cane. Marggraf tried to develop the isolation process, but his pupil, Franz Achard, realised that the yield of sugar extracted was low and set about breeding sweeter crops of beet. Progress was initially very slow, but in 1774 he began small-scale production. Finally, in 1801 he opened a factory in Silesia. This never made a profit, but the solvent extraction technique was now firmly established. When cane sugar supplies from the West Indies could no longer enter mainland Europe due to Napoleon’s ban on trade with Britain, Benjamin Delessert opened a factory in Paris to produce beet sugar in
1813, much to the delight of the Emperor. An acute awareness of the importance of solvent extraction now existed among chemists in the city.

The Swedish chemist Carl Wilhelm Scheele (1742–1786) had been the first to apply the process of solvent extraction in the isolation of acids from plant juices. He also exploited the solubility of substances of varying polarity in polar and non-polar solvents whereby polar impurities were left behind in water when it and an immiscible organic solvent such as ether were shaken together in a vessel from which the aqueous layer could be removed after the immiscible layers separated on standing. Once the remaining solvent had been evaporated, the crude residue was purified by crystallisation. This enabled Scheele, during the 1780s, to isolate citric, malic, oxalic and gallic acids. He only attempted to isolate organic acids, possibly because of the prevailing impression that active principles from plants would be acids.

So successful was solvent extraction that it still remains a powerful tool in the hands of the natural product chemist. However, since the quest to obtain penicillin during the Second World War, it has been complemented by chromatographic techniques that exploit the divergent abilities of different molecules to adhere to materials such as silica gel or dried alumina in the presence of solvents of varying polarity. Columns and thin layers of adsorbent materials have been used for both qualitative and quantitative separation of natural products, supplemented in recent times by preparative high-performance liquid chromatography.

Once a product had been isolated it had to be subjected to testing in order to ensure that it was indeed an active principle. For Scheele, this presented no problem since he isolated fruit acids with instantly identifiable taste and which were not harmful, but when later experimenters chose to test the material on themselves it was not without risk! Chemists recognised they were isolating potent poisons and soon switched to testing their extracts on animals. It is no coincidence that within 30 years of the initial isolation of active substances from plants, experimental pharmacology had begun to develop as a distinct discipline in the hands of Rudolf Buchheim (1820–1879). He was appointed to the first-ever university Chair of Pharmacology at Dorpat in Estonia when he was only 27 years old. Although it was then an Imperial Russian university, the language of instruction and most of the staff were German. Buchheim then built and paid for the first pharmacology laboratory in the world in the basement of his own home. By 1860 his reputation was such that the university finally provided him with a large purpose-built institute. He was succeeded in 1869 by his former student Oswald Schmiedeberg, a son of a local forester, who had obtained his doctorate three years earlier. Schmiedeberg moved to the newly re-established University of Strassburg (as it was then known), which opened when the city was returned to Germany after the Franco-Prussian War of 1870–1871. No expense was spared by the Reichsland of Elsass-Lothringen (now Alsace-Lorraine) in turning its university into one of the finest in Germany. Schmiedeberg was able to build a magnificent Institute of Pharmacology which took until 1887 to complete. His research proceeded unhindered, enabling Schmiedeberg to persuade the best students to work with him at a time when elsewhere pharmacology was held in low esteem. During the 46 years Schmiedeberg occupied his chair, he trained most of those who were to become professors of pharmacology at German and foreign universities. His endeavours led directly to the overwhelming pre-eminence of the German pharmaceutical industry from the 1880s until the outbreak of the Second World War in 1939.

OPIUM ALKALOIDS

Opium (Greek: *opos* = juice) is obtained by drying the latex that exudes from the capsule of the poppy, *Papaver somniferum* L. The suggestion that the *Ebers Papyrus* refers to a mixture of
poppy pods and flies as a sedative for children must be questioned in the light of a recent chemical investigation of material from the tomb of an Egyptian royal architect, which has thrown doubt on claims that the opium poppy was known in Ancient Egypt around the time of the compilation of the *Ebers Papyrus*. In the *Iliad*, Homer mentions poppy growing in gardens, implying that their cultivation had been established by the eighth century BC. Later, Aristotle stated that the poppy was narcotic since its vapours rose to the brain.

The first clear description of the poppy appeared in the *Historia Plantarum* by Theophrastus. The collection of opium from the plant is mentioned, but the earliest accurate account of the procedure is to be found in the *Compositiones* written by Scribonius Largus around AD 43. Pliny the Elder (23–79) also mentioned opium, in his 37-volume work on natural history. Sharing his fellow citizens’ contempt for Greek physicians who monopolised medical practice in Rome, he did not lose the opportunity to warn of its dangers. Nonetheless, its pain-relieving properties were clearly established and opium was prescribed by Galen (129–199). Alexander of Tralles also mentioned it repeatedly in his *De Re Medica*, written in the middle of the sixth century. Macht has written an account of the subsequent history of opium.

In 1803, Charles Louis Derosne, owner of a fashionable Parisian pharmacy in the Rue St Honoré, delivered a memoir to the Société de Pharmacie, in which he reported that in the course of devising an assay for opium he had isolated a novel crystalline salt. A variety of tests revealed that it had alkaline properties, which Derosne attributed to contamination by the potash used to precipitate it from acid solution. He appreciated that he had been handling a peculiar substance that was not a plant acid, but he described it as a salt simply because it readily crystallised. He added the rider that this was a circumlocution to compensate for his inability to assign it to any known class of chemical compounds. The matter rested there.

Armand Séguin, director of a successful tannery outside Paris, reported to the Institut de France in December 1804 that he had isolated a novel plant acid and also a crystalline narcotic from opium. His findings were presented in a paper that he submitted to the Académie des Sciences, but it was laid aside. Séguin did not continue with his investigations, and by the time his paper finally appeared in the *Annales de Chimie* in 1814 similar observations had been reported elsewhere.

Friedrich Wilhelm Sertürner (1783–1841), the son of an Austrian engineer in the service of Prince Friedrich Wilhelm of Paderborn and Hildesheim, completed his apprenticeship with the court apothecary in 1803 when he was 20 years old. He remained at the Adlerapotheke in Paderborn for a further 30 months, during which period he was able to carry out a variety of chemical experiments. Examining opium, he was quickly able to extract an organic acid that had not been reported in the literature. He named it meconic acid (Greek: *mekon* = poppy). When tested on dogs, it was inactive. However, alkalisation of the mother liquors with ammonia caused precipitation of a substance that he crystallised from alcohol. When he administered this to a dog, it induced drowsiness. He published a preliminary report in 1805 in Johann Trommsdorff’s *Journal der Pharmacie*. A detailed account of his isolation of the ‘principium somniferum’ appeared the following year. Sertürner included in his paper a footnote stating that he had not been aware of Derosne’s work until after his own had been completed. That he also wrote of the ‘almost alkali-like character’ of this principle from a plant source should have caught the attention of chemists, for all plant principles isolated prior to this were acidic in nature. That his work failed to arouse interest was due to his isolation and the fact that he published his findings in a journal read mainly by practising apothecaries.

Sertürner had explained that his ‘principium somniferum’ could neutralise free acid, yet he did not recognise the significance of this aspect of his work. It was another three years before he briefly renewed his investigations after opening his own apothecary in Einbeck, Westphalia. In 1811, he published further papers in Trommsdorff’s *Journal der Pharmacie*, in which he...
confirmed that the narcotic principle of opium was an alkaline substance, or base, that formed salts with acids. However, it was not until 1815 that he carried out a more detailed investigation on it in the Einbeck Ratsapotheke and identified the presence of carbon, hydrogen, oxygen and possibly nitrogen. In 1817, he published yet another paper, but this time it appeared in a prominent journal, Gilbert’s *Annalen der Physik*. Sertürner drew attention to the particular ease with which what he called morphium reacted with acids to form readily crystallisable salts. He also described how he and three youths swallowed doses of about 100 mg and experienced the symptoms of severe opium poisoning for several days, despite recourse to strong vinegar to induce vomiting when these symptoms first appeared! This time, Sertürner was not ignored. Joseph Gay-Lussac, the doyen of French chemists, read the paper and immediately had it translated and republished in the prestigious *Annales de Chimie*, the journal founded by Lavoisier in 1789 and which Gay-Lussac now edited.

Gay-Lussac wrote an editorial to accompany the translation of Sertürner’s paper. In this, he expressed surprise that Sertürner’s work had been ignored for so many years, but not simply because the isolation of the active principle of opium was important. Of much greater significance, according to Gay-Lussac, was the discovery of a salt-forming organic plant alkali analogous to the familiar organic acids. He predicted that many other organic alkalis would be found in plants, for there was already some evidence to suggest that the few crude active principles isolated in the previous decade contained nitrogen and had alkaline properties. To ensure a degree of conformity in the naming of plant bases, Gay-Lussac proposed that their names should end with the suffix ‘-ine’. This was the first time such standardisation of nomenclature had been suggested in organic chemistry. For this reason, Gay-Lussac altered Sertürner’s term ‘morphium’ to ‘morphine’. In 1818 the German chemist Wilhelm Meissner introduced the term ‘alkaloid’ to describe plant alkalis, though several years passed before it was generally accepted.

Gay-Lussac asked Professor Robiquet at the Ecole Supérieure de Pharmacie to check Sertürner’s experimental work. Robiquet noted the differences in the properties of the salts isolated by Derosne and Sertürner, and concluded that they were different plant alkalis. He then purified the base isolated by Derosne and gave it the name ‘narcotine’, but despite this it had no narcotic properties. It was later found to retain the cough suppressant properties of morphine, and is still prescribed for this purpose under the name of ‘noscapine’.

It subsequently transpired that even the purest samples of morphine had been contaminated with narcotine and it was not until 1831 that William Gregory, Professor of Chemistry at the University of Edinburgh, devised an economic process for obtaining the alkaloid in a high state of purity. The following year he prepared pure morphine hydrochloride, which a local laudanum (i.e. tincture of opium) producer, John Macfarlan, immediately began to manufacture in bulk. Morphine hydrochloride gained considerable importance after Alexander Wood, also of Edinburgh, developed the hypodermic syringe in 1853 for administering subcutaneous injections. The quality of pain relief obtained by patients was thereby greatly enhanced.
The chemical structure of morphine was not elucidated until 1923, when J. Masson Gulland and Robert Robinson at the University of St Andrews in Scotland succeeded in establishing it correctly. Morphine was synthesised in 1950 by Marshall Gates and Gilg Tschudi at the University of Rochester, New York, but their complex synthesis was purely of academic interest. This has proved to be the case with most alkaloids. Despite its addictive liability and hazardous depressive effect on the respiratory centre plus a tendency to cause nausea and constipation, morphine still remains the most effective drug available for the alleviation of severe pain. Its analgesic power is enhanced by the associated state of euphoria and detachment.

Three more opium alkaloids were eventually isolated from the mother liquors remaining after the extraction of morphine from opium. Thebaine was isolated in 1832 by Thibouméry, the manager of the factory built by Pelletier to produce alkaloids. It was of no medicinal value, yet it eventually turned out to be a key compound for chemists since the reactivity of its conjugated diene system permitted the synthesis of novel codeine (and morphine) analogues from it.

In the same year, Pierre-Jean Robiquet stumbled upon another new alkaloid while examining an alternative morphine extraction procedure. He named it codeine (Greek: κοδεία = poppy capsule). Although its narcotic properties were mild, it retained the full capacity of morphine as a cough suppressant. This property of opium had long been recognised, being cited in the fourteenth century by Actuarius of Constantinople. Magendie considered the main value of codeine was as a cough suppressant, especially in whooping cough. To this day, codeine linctus remains a popular remedy for coughs, but it is also a popular ingredient in combination with aspirin or paracetamol for the relief of mild to moderate pain.

It was not until 1848 that George Merck, the son of an alkaloid manufacturer in Darmstadt, Germany, isolated papaverine from the mother liquors remaining after the extraction of morphine. As it exhibited only feeble analgesic activity it was ignored until after the American pharmacologist David Macht, in 1916, described its spasmolytic action on smooth muscle. From then until the introduction of synthetic atropine analogues in the 1930s, it was frequently prescribed as a vasodilator and antispasmodic.

**EMETINE**

Shortly before the French translation of Sertürner’s 1817 paper on morphine was published, one of the professors at the Ecole Supérieure de Pharmacie, Pierre-Joseph Pelletier, turned his attention to the isolation of plant alkaloids. Impure crystals that he obtained from ipecacuanha root were given the name ‘emetine’ and were tested on animals by François Magendie, who subsequently recommended their use in the treatment of amoebic dysentery. Pelletier prepared purer crystals in 1823, but these were later found to consist of a mixture of several related alkaloids, all of which were less effective.
The pure alkaloid was not obtained until 1887. Robert Robinson determined the structure of emetine in 1948 and the alkaloid was synthesised in 1952 by R.P. Evstigneeva and his colleagues at the M.V. Lomonossov Chemical Technical Institute in Russia. A chiral synthesis was independently achieved later that decade.

QUININE

Concern about the variable responses of his patients to treatment with cinchona bark led the Portuguese surgeon Bernardino Gomez to attempt to isolate the active principle. In 1811, he managed to obtain silvery crystals by first extracting the bark with dilute acid and then neutralising it with alkali. He named the crystals ‘cinchonin’ (later changed to cinchonine), and assumed they were the sole active principle. They did not possess the bitter taste of cinchona. Gomez noted that the chemical properties of cinchonin were unlike those of any known plant product, but he did not have any reason to believe that it was a plant alkal. It was only after Gay-Lussac had drawn attention to the existence of such alkalis that cinchona bark was carefully examined for their presence.

In 1820, Pelletier and Joseph-Bienaimé Caventou repeated Gomez’s experiments on cinchona. By modifying the procedure somewhat, they isolated not only cinchonine from samples of grey bark, but also a more potent alkaloid, quinine, from yellow bark (Cinchona cordifolia). In their paper of 1820 announcing their isolation of quinine, Pelletier and Caventou urged medical practitioners to study the therapeutic properties of the pure cinchona alkaloids. This marked a new departure, for previously the active principles of plants had been isolated for analytical rather than therapeutic purposes. By urging their medical colleagues to study pure plant principles, Pelletier and Caventou set the course of drug discovery in a new direction.

Among the first to experiment with quinine was Magendie. He evaluated it in dogs and then went on to treat patients. By including it in his widely consulted Formulaire pour la Préparation et l’Emploi de Plusieurs Nouveaux Médicaments, he ensured that knowledge of quinine would quickly spread around the world. This small volume, of which there were to be many editions, listed pure chemicals rather than the hitherto standard mixtures and plant products found in similar works. This was a significant advance, for it enabled Magendie’s successors to consolidate the practice of experimental medicine on a firm basis, with the assurance that the drugs they studied were of unchanging constitution.

Magendie was so impressed with quinine and the other alkaloids that he wrote in an English translation of his Formulaire:

“We know how advantageous it is in the treatment of disease to be certain of the precise dose of all active remedies; this advantage especially applies to the present case, because the quantity of the alkalis contained in the cinchonas varies prodigiously, according to the nature
and quality of the bark which is employed. It is often also very desirable to administer this medicine in a small volume, and in an agreeable form. Patients often die of malignant fevers because they cannot swallow the necessary quantity of the bark in powder. Some throw it up after having taken it; and in others superpurgation arises, so that the powder passes through the intestinal canal without producing any effect; even in the most favourable cases it is necessary the patient’s stomach should, as it were, chemically analyse the bark with which it is filled, and extract the febrifuge principle. A process like this will be always difficult and fatiguing even for the strongest stomach. Chemistry, therefore, has done a great service to medicine, by shewing how this separation may be accomplished beforehand.”

Other Parisian physicians confirmed that quinine was able to arrest their patients’ fevers and could be used instead of the unpalatable, nauseating powdered cinchona bark. To meet the immediate demand for supplies of quinine, Pelletier and Caventou manufactured it in their own pharmacies in the Rue Jacob and Rue Gaillon. The following year, Pelletier rushed supplies of quinine to Barcelona where an epidemic had broken out. Soon, factories had to be opened, and by 1826 Pelletier’s factory was processing more than 150,000 kg of cinchona bark yearly, yielding around 3600 kg of quinine sulfate. Production elsewhere was also expanding, thanks to Pelletier and Caventou openly publishing the full details of how to obtain quinine from cinchona bark. Several manufacturers in Germany also prepared quinine. These and others quickly recognised the economies obtained through large-scale production of quinine and morphine, and also acquired specialised knowledge that enabled adulterated or inferior plant material to be rejected. In this way, the modern pharmaceutical industry gradually became established.

Soon after its introduction, quinine began to replace the vegetable products described as ‘antiperiodics’, which had been commonly used to relieve malarial paroxysms, still the commonest febrile condition at that time. This drew attention to its antipyretic properties, with the result that until the 1880s quinine became the most frequently prescribed drug for lowering temperature in any febrile condition. It was not until the end of the nineteenth century that the mode of action of quinine in malaria was revealed. At St Petersburg in 1891, Yuri Romanovsky applied a stain of eosin and methylene blue to reveal that the malarial parasites in blood samples taken from patients treated with quinine were damaged. These observations led him to the conclusion that the action of quinine was directly on the parasites rather than on the patients. This was a revolutionary concept, yet it drew little attention.

Despite its toxicity, quinine was unrivalled in the treatment of malaria until the introduction of safer synthetic drugs. These displaced it, but the problem of widespread drug resistance has resulted in the reintroduction of quinine for treatment of malaria caused by resistant strains of *Plasmodium falciparum*.

The chemical structure of quinine was confirmed by its synthesis at Harvard University in 1944 by Robert Woodward and William von Eggers Doering. 
QUINIDINE

Soon after the introduction of cinchona bark, the German physician Georg Stahl had recognised its action on the heart and subsequently sporadic attempts were made to exploit this. In the second half of the nineteenth century, some physicians prescribed quinine on account of its depressant action on heart muscle. In 1912, a patient told the Dutch cardiologist Karl Wenckebach that he took quinine to bring his atrial fibrillation under control. When Wenckebach tried quinine on other patients, only one responded favourably. In his book on cardiac arrhythmias, published in 1914, Wenckebach referred to the matter in passing. Four years later, a leading Viennese medical journal carried a report from Berlin by Walter Frey, which stated that quinidine was the most effective of the four principal cinchona alkaloids in controlling atrial arrhythmias. Quinidine was also the least toxic, but was still a hazardous drug requiring skilled administration. It was an optical isomer of quinine isolated in 1833 by L. Henry and Auguste Delondre and was found to be inferior to quinine as an antimalarial.29 It is still used for suppression of supraventricular and ventricular arrhythmias.

XANTHINE ALKALOIDS

The stimulating effects of coffee, Coffea arabica L., have long been recognised in the Middle East. Coffee was introduced into England and France from Constantinople in the middle of the sixteenth century and became a popular beverage. Ferdinand Friedlieb Runge isolated a stimulant alkaloid from mocha beans given to him by the German poet and chemist Goethe in 1821 and named it caffeine.30 The chemical structure was established by Emil Fischer at the University of Berlin in 1882. He also synthesised it three years later.31 Caffeine soon became popular as a stimulant. Until the introduction of the synthetic diuretics, it and the other xanthine alkaloids present in tea, cocoa and coffee were frequently prescribed as diuretics until the introduction of the thiazides. Theobromine, isolated in 1878 from seeds of the cacao tree, Theobroma cacao L. (Sterculiaceae), was found to be more effective than caffeine as a diuretic, while less stimulating.32 Theophylline from the tea plant, Camellia sinensis L. (Theaceae), that grows in India and China was isolated in 1888.35 Its chemical structure was elucidated in 1895.31 As the yield of alkaloid was only 0.1% it was not evaluated clinically until after its synthesis. In 1902, Oskar Minkowski established that it was around three times as potent a diuretic as caffeine.

In a widely read treatise on the treatment of asthma published in 1860, the London physician Henry Salter noted that strong black coffee could relieve the breathing problems of asthmatics.34 It was later shown that theophylline was a bronchodilator. However, the safety margin of theophylline in the treatment of asthma is quite narrow and it has been largely superseded by modern bronchodilators.

ATROPINE AND HYOSCINE

Country people have long been aware that belladonna, Atropa belladonna L. (Solanaceae), can dilate the pupil, but it was a study by Karl Himly in Paris in 1802 that encouraged ophthalmologists to use belladonna to assist examination of the eye.35 An impure alkaloid was
obtained in 1822 by Rudolph Brandes, pure atropine being isolated from the roots by Philipp Geiger in 1833. Atropine is an artefact formed during the extraction of the alkaloid from the plant. The naturally occurring material is hyoscyamine, present in plants as a laevorotatory stereoisomer. During isolation, hyoscyamine transforms into a mixture of equal parts of its two optical isomers. This mixture is atropine, and is the form in which the alkaloid is used clinically, even though most of the activity resides in the laevo-isomer.

Several plants of the family Solanaceae contain both hyoscyamine and a structurally similar alkaloid known as hyoscine. Both alkaloids block the access of the neurohormone acetylcholine to the receptor site where it acts once it is released from cholinergic nerve endings. In the United States, hyoscine is known as scopolamine, reflecting the use of Scopola carniolica as a source of this mydriatic alkaloid. This plant is so named because it was studied at the University of Pavia by the eighteenth century botanist Johann Scopoli.

There is a long history in Ayurvedic medicine of the inhalation through a hukka of the vapour produced by warming Datura ferox L. leaves as a means of relieving asthma. Dr James Anderson, Fellow of the Royal Society and Physician General at the Madras Hospital in India, was an enthusiastic botanist who benefited from this treatment in 1802 and duly reported it to his friend Dr Sims in Edinburgh. Following the publication in 1812 of a report in the Edinburgh Medical and Surgical Journal by Dr Sims, cigarettes containing the leaf of D. stramonium became widely used by asthmatics. After a report by Henry Salter in 1869 describing the successful administration of belladonna and also atropine in asthma, the latter became widely used for this purpose. Other preparations containing the leaf were also used in the nineteenth century, including the highly popular Potter’s Asthma Cure.

Rudolf Buchheim believed that the sedative properties of henbane might be due to the presence of a second alkaloid besides hyoscyamine, but it was Alfred Ladenburg at the University of Kiel who isolated hyoscine in 1881 and showed that it was structurally similar to atropine.

Workers in the Hoffmann–LaRoche laboratories in Switzerland overcame a major impediment to the clinical acceptibility of hyoscine preparations when they discovered that transformation of the alkaloid took place in solution to form equal amounts of its two optical isomers. The dextrorotatory isomer was shown to be a central nervous system stimulant, in contrast to the laevorotatory isomer, which was a sedative. This explained why the racemic combination produced unpredictable effects. It became possible to use the laevorotatory isomer of hyoscine for pre-anaesthetic medication in formulations such as papaveretum and hyoscine injection, introduced in 1910.

**PHYSOSTIGMINE**

William Daniell, a British Army surgeon, reported to the Ethnological Society of Edinburgh in 1846 that he had observed the use of the Calabar bean, Physostigma venenosum Balf. (Leguminosae), as an ordeal poison in Old Calabar, Nigeria. Criminals convicted of capital offences were forced to swallow a watery extract of the beans. It has been suggested that those with a clear conscience swallowed the drug, which either caused them to vomit or resulted in...
hydrolysis of the active principle, whereas those who were guilty retained it in their mouth and thus facilitated buccal absorption.

Daniell's report interested Robert Christison, Professor of Materia Medica at Edinburgh, who arranged for a missionary to send him a Calabar bean, which he then had cultivated. Animal experiments showed that extracts of the bean killed by stopping the heart. Uncertain about the authenticity of the bean he had received, Christison experimented on himself. He experienced extreme weakness and was fortunate to survive, for his heart had probably gone into atrial fibrillation. His distinguished student Thomas Fraser isolated the active principle as an amorphous powder. Jobst and Hesse in Germany obtained pure crystals in 1864 and named their product 'physostigmine'.

The value of physostigmine in preventing blindness caused by glaucoma was revealed in 1875 by Ludwig Laqueur. It remains in use for the treatment of chronic simple glaucoma, drops being applied to the eye several times a day to relax the ciliary muscles and improve outflow of aqueous humour with consequent reduction in the elevated intraocular pressure.

**COCAINE**

The earliest written account of the coca bush, *Erythroxylon coca* L. (*Erythoxylaceae*), appeared in Nicolas Monardes' *Historia Medicinal de Indias Occidentales*, published in Seville in 1565. To the Indians of Peru it was the incarnation of a god, with the fields where it grew being revered for their holiness. Its use, however, was restricted to only the most noble of the Incas until the Spanish authorities introduced legislation at the second Council of Lima, in 1569, making the plant freely available to all. The subsequent popularity of the coca leaf among the Indians was described by William Prescott in 1847 in his *History of the Conquest of Peru*. This stimulated interest in coca leaves among organic chemists, culminating in the isolation of pure crystals of cocaine in 1860 by Albert Niemann at Göttingen University.

During the next two decades, cocaine was considered to be a mild stimulant similar to caffeine. Parisian society imbibed Vin Mariani, a medicinal wine first prepared from an infusion of coca leaves by Alberto Mariani in 1863. The popularity of this and similar beverages spread to the United States, where even the medical profession lauded Mariani Elixir, Mariani Lozenges and Mariani Tea! One of the ingredients when Coca-Cola was introduced in the United States in 1886 was coca leaf, until the formulation was changed in 1903.

A report that cocaine had helped Bavarian soldiers to withstand hunger and fatigue during taxing military manoeuvres led Sigmund Freud to investigate whether cocaine could counter fatigue, his findings later being published as *The Cocaine Papers*. He was assisted by Carl
Koller, a trainee ophthalmologist. Koller knew that anaesthesia with ether or chloroform was hazardous in eye surgery as patients could vomit or be restless on awakening. He had experimented with various drugs such as chloral hydrate, sodium bromide and morphine as potential topical anaesthetics for the eye, but met with no success. It was therefore fortuitous that in the course of one of Freud’s experiments a volunteer told Koller that cocaine numbed his mouth. Although a recognised characteristic of the alkaloid, Koller now appreciated its significance for his work. Returning to the Vienna General Hospital, he was met by his professor’s assistant, who later published his recollection of what happened after Koller produced a small flask containing cocaine hydrochloride:

“A few grains of the powder were dissolved in a small quantity of distilled water, a large, lively frog was selected from the aquarium and held immobile in a cloth, and now a drop of the solution was trickled into one of the protruding eyes. At intervals of a few seconds the reflex of the cornea was tested by the touching of the eye with a needle. . . . After about a minute came the great historic moment. I do not hesitate to describe it as such. The frog permitted his cornea to be touched and even injured without a trace of reflex action or attempt to protect himself – whereas the other eye responded with the usual reflex action to the slightest touch. With the greatest, and surely considering the implications, most justifiable excitement, the experiment continued. The same tests were performed on a rabbit and on a dog with equally good results.

Now it was necessary to go one step further and repeat the experiment upon a human being. We trickled the solution under the upraised lids of each other’s eyes. Then we put a mirror before us, took a pin in hand, and tried to touch the cornea with its head. Almost simultaneously we could joyously assure ourselves, ‘I can’t feel a thing!’ We could make a dent in the cornea without the slightest awareness of the touch, let alone any unpleasant sensation or reaction. With that, the discovery of local anaesthesia was completed.”

Koller’s findings were presented to a meeting of the Ophthalmological Society in Heidelberg on 15 September 1884. Within weeks, cocaine anaesthesia was introduced in Europe and the United States and had a far-reaching impact on surgical practice. Because of its widespread abuse, it is now used medicinally only as a topical anaesthetic for the nose, throat and larynx. For other purposes it has been superseded by synthetic local anaesthetics.

**PILOCARPINE**

A drug frequently employed to mimic the action of acetylcholine on the eye is pilocarpine, an alkaloid obtained from the South American shrub *Pilocarpus jaborandi* Holmes (Rutaceae). Symphronio Coutinhou, a Brazilian physician, observed that the leaves caused excessive salivation when chewed by natives and took samples with him on a visit to Europe in 1873. Pilocarpine was isolated the following year by A.W. Gerrard in London and, independently, by E. Hardy in France.

Pilocarpine was so effective as a diaphoretic in the treatment of oedema that it extended the scope of this type of therapy until the introduction of potent diuretics in the 1920s. In 1877, Adolf Weber discovered its value in decreasing intraocular pressure in glaucoma. Nowadays, it is used solely for this purpose. Pilocarpine is one of the few examples of a natural product...
from which analogues have not been developed, principally because of its inherent potency and acceptability for the use to which it was put.

CURARE

Pietro Martire d’Anghiera, Royal Chronicler to Charles V of Spain, published *De Orbe Novo* in 1516. Describing the experiences of the first travellers to return from the New World, he wrote of a soldier being mortally wounded by an arrow tipped with poison. Further details of this poison emerged in over 50 other reports by visitors to South America during the sixteenth and seventeenth centuries.51 One written by Laurence Keynes, who served with Sir Walter Raleigh on his expedition to the region now known as Venezuela, featured a list of poisons that included a herb known by the natives as ‘ourari’. From this term arose the word ‘curare’ to describe South American arrow poisons that cause paralysis.

In 1735, the French Academy of Sciences sponsored an expedition to Ecuador in which Charles Marie de la Condamine was to conduct topographical studies that would determine the length of a degree of meridian at the equator and thus establish how much the planet flattens towards the poles. During the eight years he remained there he collected samples of curare that could kill chickens when injected. In 1850, Claude Bernard reported that even though curare prevented the ligated limb of a frog from responding to nerve stimulation, both the nerve and muscle retained their ability to function.52 He concluded that curare acted at the neuromuscular junction to interrupt the stimulation of the muscle by the nerve impulse. In 1936, Dale, Feldberg and Vogt at the National Institute for Medical Research in London showed that acetylcholine was the chemotransmitter at the neuromuscular junction and that curare owed its effects to antagonism of it.53

Sporadic attempts to employ curare in the treatment of rabies, tetanus, chorea and epilepsy met with little success during the nineteenth century. The extracts were of dubious quality and variable potency due to the uncertainty that surrounded the source of the plant from which they were made. It was not until 1939 that a firm identification of the source of a physiologically active curare preparation was made by Richard Gill, a coffee and cacao plantation owner in Ecuador who spent five months collecting 26 varieties of lianas (vines), from which he prepared over 50 kg of curare.54 Gill supplied a large quantity of his curare to Abram Bennett, a neuropsychiatrist practising in Nebraska, who had been trying to obtain samples of curare to cure vertebral fractures during the newly developed technique of convulsive shock therapy.55 The curare was given to A.R. McIntyre of the Department of Pharmacology at the University of Nebraska for standardisation.56 Bennett then carried out a clinical trial of the curare on 12 spastic children at the Nebraska Orthopedic Hospital. The relaxing action of curare proved to be transient, but the results in shock therapy were much more encouraging. It rendered this otherwise dangerous therapy for depressive patients safe enough for routine application.

Gill signed a contract with E.R. Squibb & Sons, who purchased his remaining supply of curare. Horace Holaday then simplified the standardisation procedure and Squibb made curare available free of charge to clinical researchers as Intocostrin®, which they described as ‘unauthenticated extract of curare’.

The use of curare to permit pelvic examinations on disturbed female patients gave Lewis Wright, a medical consultant to Squibb, the idea that it might be possible to administer curare to overcome the lack of adequate muscle relaxation associated with recently introduced anaesthetics such as ethylene and cyclopropane. He believed it might also permit adequate relaxation with lighter levels of older anaesthetics such as ether and chloroform. Unfortunately, when Intocostrin® was administered to cats and dogs anaesthetised with ether, the cats died and the dogs became deeply distressed. Wright persuaded Harold Griffith, a part-time anaesthetist at the Homeopathic Hospital in Montreal, to test Intocostrin® on
patients anaesthetised with cyclopropane. Since Griffith kept his patients on artificial respiration when administering cyclopropane because of the risk of respiratory depression, he unwittingly overcame the two problems with Intocostrin® in anaesthesia. Firstly, ether had neuromuscular blocking activity in its own right, thereby potentiating the effects of the Intocostrin®. Secondly, paralysis of the respiratory musculature by Intocostrin® made artificial respiration a necessity. The outcome of a test of the drug in a youth undergoing an appendectomy encouraged Griffith to inject curare in a further 25 patients. This and other clinical trials confirmed the role of the drug in making anaesthesia safer by permitting low doses of anaesthetic while still attaining muscular flaccidity.

Gill’s particular sample, which had provided the highest yield of curare for Intocostrin® production, was identified as *Chondodendron tomentosum* Ruiz et Pavon (Menispermaceae) at the New York Botanical Gardens. In 1943, Squibb chemists Oskar Wintersteiner and James Dutcher® isolated crystals from this which proved to be identical to those obtained eight years earlier by Harold King at the National Institute for Medical Research in London from a preparation of curare obtained from the British Museum.® The botanical source of that preparation was unknown, but since the material had been packed in a bamboo tube, King had named it tubocurarine.

The isolation of tubocurarine from *C. tomentosum* coincided with the completion of the clinical trials on Intocostrin®, enabling pure tubocurarine chloride to be introduced into anaesthetic practice. As it paralysed respiratory as well as all voluntary muscles, anaesthetised patients had to be artificially ventilated until its long-lasting effects wore off. Newer analogues have generally superseded tubocurarine since they have a shorter duration of action.

**EPHEDRINE**

Ma Huang, a mixture of *Ephedra sinica*, *E. equisetina* and *E. gerardiana* (Ephedracea), is well known in Chinese medicine. Li Shih-Chen in the late sixteenth century herbal *Pen Ts’ao Kang Mu* claimed that it could improve the circulation, cause sweating, ease coughing and reduce fever. An impure alkaloid was extracted from it in 1885 by G. Yamanashi at the Osada Experimental Station. His work was continued by Nagajosi Nagai who isolated it at Tokyo University and named it as ‘ephedrine’®. Nagai also elucidated its structure and asked a colleague to examine its physiological properties. These were reported to be comparable to those of atropine, with the advantage that the mydriatic effect lasted only two hours. It was employed in Western medicine to dilate the pupil, but was considered too toxic for other applications. Ephedrine was synthesised by Spáth in 1920.®
Unaware of these earlier studies, Ku Kuei Chen and Carl Schmidt investigated extracts of Ma Huang at Peking Union Medical College in 1923. A decoction injected into a dog caused a sustained rise in blood pressure, accompanied by an accelerated heartbeat and constriction of the blood vessels in the kidney. These effects were similar to those of adrenaline and tyramine. Chen then used solvent extraction techniques to isolate crystals of the alkaloid previously obtained by Nagai. Further tests demonstrated that, unlike adrenaline, ephedrine was effective by mouth and long-acting. Recognising the clinical potential of this, Chen and Schmidt supplied some ephedrine to Thomas G. Miller at the University of Pennsylvania, who investigated its potential in asthma, and L.G. Rowntree at the Mayo Clinic. Their clinical investigations culminated in the approval of the drug by the Council of Pharmacy and Chemistry of the American Medical Association in 1926. It rapidly became one of the most widely used drugs in the pharmacopoeia because of its value in asthma, where the prolonged action on the bronchi helped to ward off attacks of bronchospasm. Ephedrine has been superseded by drugs such as salbutamol and terbutaline, but is still prescribed as a nasal decongestant. Pseudoephedrine is a naturally occurring stereoisomer of ephedrine with similar properties. It is used as a decongestant in proprietary cough mixtures and nasal drops.

RAUWOLFIA ALKALOIDS

In the early years of the twentieth century, the British colonial authorities in India were concerned about the failure of Western medicine to make inroads into local practice. Committees of Inquiry investigated Indian herbal remedies such as the snakeroot plant, so called on account of its appearance having led to its use as an antidote to snakebite. The earliest mention of snakeroot appeared in the Charaka Samhita, a revered Ayurvedic treatise probably written around the first century AD. In 1563, it was described as 'the foremost and most praiseworthy Indian medicine' by the Portuguese physician Garcia de Orta (1500–1569) in his work about Hindu medicine, Cloquios dos Simples e Drogas e Consas Medicinais da India. The French botanist Charles Plumier in 1703 named the plant Rauwolfia serpentina (Apocynaceae) after the German explorer and botanist Leonhard Rauwolf, who had written a description of it in 1582. More than 30 applications were known early that century in India, yet no serious investigation of it took place until 1931.

In 1931, Salimuzzaman Siddiqui and Rafar Hussein Siddiqui at the Research Institute for Unani–Ayurvedic Medicine in Delhi isolated an alkaloid from snakeroot which they named ‘ajmaline’, after the physician–wizard Hakim Azmal Khan who founded the Institute. Another five alkaloids were later isolated, but none of them exhibited any properties of interest when tested on frogs. Meanwhile, Gananath Sen and Kartick Bose in Calcutta reported that rauwolfia not only reduced blood pressure if administered for a few weeks, but it also produced sedation. This vindicated the folk use in Bihar where it was given to put babies to sleep and to calm violent lunatics.

Ram Nath Chopra and his colleagues at the School of Tropical Medicine in Calcutta spent more than ten years investigating the pharmacology of rauwolfia and its alkaloids. They confirmed its ability to reduce blood pressure and, although they also demonstrated that crude extracts of the root had powerful sedative properties, they were unable to isolate an alkaloid with sedative activity. Nevertheless, around one million Indians received rauwolfia for their high blood pressure during the 1940s.

There was no interest in rauwolfia in the West until 1949, when Rustom Jal Vakil published the findings of a controlled clinical trial of the effects in 50 patients treated over a five year period at the King Edward Memorial Hospital in Bombay. His results indicated the beneficial effects of the therapy and caught the attention of Robert Wilkins, director of the hypertension clinic at Massachusetts General Hospital. He persuaded E.R. Squibb & Sons to obtain rauwolfia for him to test on patients and then reported his findings in 1952, confirming
the mild hypotensive activity of the powdered root and drawing attention to its atypical sedative effect, which caused patients to feel relaxed rather than drowsy. By a fortunate coincidence, the isolation of the long sought active principle responsible for these therapeutic effects was reported around the same time.

In 1949, Emil Schlittler and Johannes Müller of the Ciba research division in Basle investigated the sedative principle of rauwolfia believed to be present in the resinous material remaining in the mother liquors of extracts from which large amounts of ajmaline had already been harvested. Their task was complicated by the presence of a wide variety of pharmacologically active compounds, some of which antagonised the action of the compound they were attempting to isolate. A further complication was the slow onset of the sedative action. Hugo Bein overcame this difficulty by selecting from the varied physiological effects of the extracts one that seemed to be present in most active fractions, namely the miotic effect on the eye of a hare. This was used to identify the best method for recovering a sparingly soluble alkaloid, which was eventually isolated in 1951. This was named ‘reserpine’, and it was soon confirmed as responsible for most of the hypotensive and sedative activity of rauwolfia root. In November 1953, Ciba began to market reserpine. It was synthesised in 1956 by R.B. Woodward at Harvard.

For several years reserpine was widely prescribed both as a hypotensive agent and as a tranquilliser, but its popularity waned. The effect on blood pressure when given by mouth proved inferior to that of new synthetic drugs, while there was a risk of precipitating a severe depression that could lead to suicide in some patients. Consequently, neither reserpine nor its semi-synthetic analogues are prescribed any longer in the United Kingdom.

VINCA ALKALOIDS

The species of periwinkle known as Catharanthus roseus (L.) G. Don. (Apocynaceae), formerly described as Vinca rosea, was indigenous to tropical zones but its attractive appearance led to its cultivation as an ornamental plant. There have been many reports of its use in folk medicine, especially in the treatment of diabetes. In 1949, Ralph Noble at the University of Western Ontario found extracts had no effect on blood sugar levels in rats either by mouth or by injection, but in the latter case the rats succumbed to infections. He then examined the blood of one rat and found that the number of white cells was dramatically reduced. He established that this was due to bone marrow damage, a phenomenon previously associated with the antileukaemic drugs then being introduced.

Noble and Charles Beer separated a pure alkaloid, vinblastine (originally known as ‘vincaleukoblastine’), in 1958. Gordon Svoboda of Eli Lilly then informed them that his company had followed a similar path after examining C. roseus for antidiabetic activity. On submitting extracts of the plant to a general pharmacological screening programme, the activity against a transplanted acute lymphocytic leukaemia in the mouse was revealed, resulting in its use in lung and breast cancer.
In 1961, Svoboda announced the isolation of vincristine, another alkaloid with promising activity. Although vincristine differed from vinblastine solely in having a formyl group instead of a methyl group attached to the nitrogen of one of the indole rings, its toxicity and range of clinical applications was different. It is used mainly to treat leukaemias and lymphomas, especially for the induction of remissions. After the introduction of these two alkaloids, the Lilly chemists isolated many more alkaloids, but none of these attained clinical acceptability. However, two semi-synthetic analogues, vindesine and vinorelbine, are used in certain cases of advanced breast cancer. Like the natural vinca alkaloids, they act by binding to the microtubules that form the spindles to which chromosomes are temporarily attached during cell division. This results in mitosis (cell division) being blocked during metaphase.

CAMPTOTHECIN

While seeking plant sterols that could be converted to cortisone, Monroe Wall of the US Department of Agriculture extracted material from the Chinese tree *Camptotheca acuminata* Decsne in 1958. As a matter of routine, this was sent to the National Cancer Institute where it was screened by Jonathan Hartwell and found to have antitumour activity. Wall and Mansukh Wani then isolated the active compound, camptothecin. Unfortunately, it turned out to be too toxic when tested in the clinic in the early 1970s. Subsequent investigations confirmed that the cytotoxic action arose from damage to both DNA and RNA.

Although camptothecin found no place in the clinic, its analogues topotecan and irinotecan are now routinely used.

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In 1763, Revd Edward Stone of Chipping Norton in Oxfordshire sent a letter to the Royal Society reporting that he had found a locally available substitute for the expensive, imported cinchona bark.1 He wrote,

"About six years ago, I accidentally tasted willow bark and was surprised at its extraordinary bitterness; which immediately raised me the suspicion of its having the properties of the Peruvian bark. As this tree delights in a moist or wet soil, where agues chiefly abound, the general maxim, that many natural maladies carry their cures along with them, or that their remedies lie not far from their causes, was so very apposite to this particular case, that I could not help applying it…"

Stone had obviously been influenced by the doctrine of signatures in this instance, but his subsequent investigation was more rational. He told the Royal Society that during the previous six years he had successfully treated 50 people with agues (malarial fevers) by giving them dried willow bark to consume.

Others had written about the uses of the bark before Stone, but he was the first to note its antipyretic properties.2 In 1792, Samuel James, a surgeon in Hertfordshire, evaluated different species of willow barks against fevers by assessing their astringent effect on the tongue.3 He found that willow bark could reduce fevers unaffected by cinchona bark. This was taken further by William White, an apothecary from Bath, who made extracts of the bark.4 There was little further interest in the use of willow as a substitute for cinchona until the English naval blockade of continental Europe during the war with Napoleon, when it became important to obtain substitutes for imported medicinal herbs.

SALICIN

A pure glycoside, salicin, was isolated in 1829 from meadowsweet, Filipendula ulmaria L. (Rosaceae), formerly known as Spirea ulmaria, by Leroux, a French pharmacist.5 Soon after, it was introduced in France as a substitute for quinine. Raffaele Piria later showed that salicin could be decomposed into salicylic acid.6 Salicin was synthesised in 1879.7

Thomas Maclagan, a Scottish physician, first used salicin in 1874 to treat a patient afflicted with rheumatic fever.8 A year and a half later, on reporting to the Lancet that he had attained successful results in around 100 patients, he also declared his conviction that there was a
similarity between intermittent fever (malaria) and acute rheumatism. Like Stone a century before, he noted that cinchona bark was to be found in tropical areas where the ravages of malaria were most severe, and believed that a remedy for acute rheumatism would be found in a low-lying damp locality with a cold climate. Maclagan felt that the plants whose haunts best corresponded to his requirements were the various forms of willow. This may have been unscientific, but his patients benefited. He was to learn that Marcellus von Nencki of Basle had shown in 1870 that salicin is converted in the body into salicylic acid. When the first reports of the antipyretic action of salicylic acid appeared, Maclagan also tried this. Again, he eased the affliction of his patients. Salicin was more expensive than synthetic salicylic acid, so its use was gradually abandoned. Nevertheless, it remained in the British Pharmaceutical Codex until 1954.

Methyl salicylate was isolated in 1843 by William Procter from oil of wintergreen, Gaultheria procumbens L. (Ericaceae), a traditional American remedy for the ague. It has been incorporated in topical anti-inflammatory and counter-irritant formulations, as well as in numerous cosmetic products.

**DIGITALIS GLYCOSIDES**

Attempts were made in the 1820s to isolate an active principle from digitalis. To stimulate research, the Société de Pharmacie in Paris offered a prize of 500 francs for the isolation of a pure principle from the plant, the sum having to be doubled after five years had passed without any claimant coming forward. In 1841, the French pharmacists E. Homolle and Theodore Quevenne won the award for their isolation of an impure crystalline material which consisted mainly of impure digitoxin. They called this ‘digitalin’, a name also applied to various products obtained by other workers.

Digitoxin, the principal cardiotonic glycoside present in the leaves of Digitalis purpurea, was isolated in 1875 by Schmiedeberg at the University of Strassburg (now Strasbourg). He obtained crystals from digitalis leaves by modifying a method employed by the pharmacist Claude Nativelle six years before for the isolation of a similar material called ‘digitalin crystallisée’. Adolf Windaus finally obtained pure digitoxin in 1925, at the University of Gottingen.

In 1920, Max Cloetta in Zurich hydrolysed digitoxin under acid conditions and isolated the aglycone, digitoxigenin, which had weak cardiotonic activity. The structure of digitoxigenin was determined by Walter Jacobs and his colleagues at the Rockefeller Institute in New York, but it was not until 1962 that chemists at Sandoz in Basle elucidated the structure of the sugar residue and hence that of the entire molecule of digitoxin.
In the late 1920s, it was discovered that the powdered leaves of *Digitalis lanata*, once popularly known as ‘woolly foxglove’, had greater physiological activity than those of *Digitalis purpurea*. This led Sydney Smith of Burroughs Wellcome in London to isolate digoxin.\(^{17}\) This is now used more than either powdered digitalis leaves or digitoxin since it does not bind as strongly to proteins in the tissues and plasma, resulting in less delay before a therapeutic concentration of unbound drug can build up. Clearance from the body is also faster as only unbound drug is filtered by the kidneys; consequently digoxin is less cumulative and thus safer to use. It has become the standard digitalis preparation in current usage.

GUAIACOL

Ascani Sobrero was an Italian chemist best known for his discovery of nitroglycerin, but he also isolated guaiacol from dry distillation of guaiac resin in 1843.\(^{18}\) After Auguste Béhal and Eugène Choay extracted crystalline guaiacol from beechwood tar in 1887, it quickly became popular as it was now free of any unpleasant smell and could even be cheaply isolated from coal tar creosote. In Berne, Hermann Sahli recommended it for the treatment of tuberculosis.

The industrial chemist Karl von Reichenbach distilled creosote from beechwood tar in 1830. Later, its physical similarity to the heavy oil distilled from coal tar led him to examine its antiseptic properties. Over the next few years numerous articles in medical journals extolled the virtues of creosote in the treatment of gangrene, cancerous lesions, eczema, impetigo and foul-smelling wounds. Pieces of cotton soaked in creosote were also applied to tooth cavities to afford genuine relief. Despite its irritant nature, beechwood creosote was even taken internally. It acquired an undeserved reputation for having extraordinary powers to arrest vomiting! Responsible medical opinion questioned this, and also claims that it could relieve diarrhoea. Creosote was also inhaled, apparently to relieve excessive bronchial secretion. Pulmonary tuberculosis was treated by inhalation of creosote combined with administration by mouth of as much as 1 g daily.
All these applications resulted in a demand for a cheap substitute for the expensive genuine beechwood creosote. This demand was often met by the supply of coal tar creosote at around one-fifth of the price. As this was contaminated with phenol it was much more irritant than genuine creosote, especially if inhaled or taken internally.

**PODOHYLLOTOXIN**

The May Apple (or American mandrake), *Podophyllum peltatum* L. (Berberiaceae), is a perennial found throughout the eastern side of North America. The juice expressed from its root was employed by the Wyandotte Indians as a laxative and anthelmintic and by the Cherokees for deafness. Podophyllotoxin was isolated from the rhizome in 1880 by Podwyssotski. Its structure was established in 1951 by Anthony Schrecker and Jonathan Hartwell at the National Cancer Institute, and a total synthesis was achieved by Walter Gensler and Christos Gatsonis at Boston University.

[Chemical structure diagram of podophyllotoxin]

John King, a physician in Ohio, pioneered the use of the highly potent podophyllum resin obtained from the juice, persuading William Merrell, a Cincinnati pharmacist, to include it in 1847 in the range of products his company supplied to the medical and pharmaceutical professions. It was sold as Podophyllin, under which name it quickly became established as one of the most popular laxatives throughout the world. The name podophyllin was accepted into pharmacopoeias, e.g. the *British Pharmacopoeia* of 1864. Today, the use of podophyllin as a laxative is discouraged since this effect is induced by severe irritation of the intestinal mucosa.

In the *Indian Doctor's Dispensatory*, published in Cincinnati in 1818, Peter Smith recommended the topical application of powdered podophyllum root as an escharotic to deal with warts because of its obvious irritant nature. This remains the principal application of both the resin and podophyllotoxin.

Podophyllotoxin was shown to be a mitotic poison, inhibiting cell division during metaphase in a similar manner to colchicine by binding to the protein fibres known as tubulin, which constitute the mitotic spindle that holds the chromosomes during the metaphase of cell division. Although this finding led to various clinical trials, podophyllotoxin has proved to be too toxic for use as a cancer chemotherapeutic agent.

It is of interest to note that Dioscorides referred to the role of oil of juniper for treating warts, carbuncles and leprosy, while Pliny the Elder mentioned that some smaller species of juniper could be used to treat tumours. The tenth century English *Leech Book of Bald* similarly recommended the root of cow parsley, *Anthriscus sylvestris* (L.) Hoffm., for cancer. Juniper contains podophyllotoxin and deoxypodophyllotoxin, while *A. sylvestris* contains the latter.

**KHELLIN**

*Ammi visnaga* (L.) Lam. (Umbelliferae) is a herbaceous plant that grows wild in Egypt, where a decoction from it acquired a reputation in folk medicine as a diuretic that could relieve renal
colic. This was a common problem in Egypt arising from renal stone formation associated with schistosomiasis. A study on a refined extract by K. Samaan at the University of Cairo in 1930 revealed its ability to relax smooth muscle, including that of the ureter and coronary arteries, in a variety of animal species. This formed the basis of a clinical investigation that confirmed that, in addition to the diuretic action, it could relieve spasm of the ureter.

Crystals isolated from the seeds by Ibrahim Mustapha at the Ecole Supérieure de Pharmacie at Montpellier in 1879 were given the name ‘khellin’ (from El Kellah, the Moorish name of the plant). In 1938, Ernst Späth obtained pure crystals at the University of Vienna and elucidated the structure. Much of the interest in khellin was directed at its action on the heart, following a serendipitous observation by Gleb von Anrep, the professor of pharmacology at Cairo, who noticed that after his laboratory technician had self-medicated his renal colic with it, his angina disappeared. Anrep then conducted tests of khellin on the coronary blood flow in dogs, followed by a clinical trial in patients with angina. This and further studies by Anrep suggested the drug had beneficial effects when given by mouth. Subsequent studies were generally in favour of the use of khellin for angina as it had a longer duration of action than had glyceryl trinitrate. It became widely prescribed until a study in the United States showed that angina patients who benefited from khellin treatment all experienced some toxic symptoms. As a result of this and similar studies, efforts were made to find less toxic analogues.

DICOUMAROL

In 1922, cattle in North Dakota and Alberta were found to be bleeding to death. A veterinary pathologist, Francis Schofield, discovered that this was caused by the eating of mouldy hay prepared from sweet clovers *Melilotus alba* Desv. and *M. officinalis* (Leguminosae). Sweet clovers had been introduced from Europe earlier in the century since they could withstand harsh winters and dry summers. After Schofield had established that the clotting time was prolonged in the sick cattle, Lee Roderick found that an unknown haemorrhagic factor caused the disease by reducing the activity of prothrombin, the precursor of thrombin. However, it was not until 1938 that progress in isolating the factor was made by Karl Link and Harold Campbell, at the Experimental Station run by the University of Wisconsin College of Agriculture. They modified a new method developed by Armand Quick for one-stage quantitative measurement of prothrombin activity in patients afflicted with various blood diseases. In 1939, Campbell isolated crystals of a potent anticoagulant. The following year, Charles Huebner confirmed by synthesis that the anticoagulant was 3,3′-methylenebis(4-hydroxycoumarin). It was formed from coumarin (which causes the smell of new-mown hay) by fungal oxidation to 4-hydroxycoumarin, followed by coupling with formaldehyde.
In 1942, the Abbott and Lilly companies marketed the new anticoagulant, which had been
given the approved name of 'dicoumarol'. Since then, numerous studies concerning the
merits or otherwise of this and related anticoagulants in thromboembolic disease have been
published. At present, the main uses of these drugs are in the prevention and treatment of deep
vein thrombosis in the legs and to prevent thrombi forming on prosthetic heart valves.
Hundreds of coumarins have been found to exhibit anticoagulant activity, the principal
differences between them concerning potency, the rate of onset of action and duration. Since
their mode of action is to interfere with vitamin K in the hepatic biosynthesis of clotting
factors, there is a time lag of 1–3 days before reserves are depleted. Coupled with a degree of
unpredictability of response, this led to dicoumarol being superseded by analogues such as
warfarin and phenindione.

TETRAHYDROCANNABINOL

Preparations of Cannabis sativa L. (Cannabidaceae) are known by many names, including
Indian hemp, marihuana, bhang, ganja, charas, kif, hashish and pot. These reflect the
widespread usage of this euphoriant plant, which occurs naturally in Asia, the Middle East,
India, and also Central and North America. Cannabis was known to the Ancient Greeks.
Herodotus described how the Scythians made clothes from its fibres and medicated their
vapour baths with its seeds. Lloyd provided a detailed list of the many references to the use of
cannabis in ancient Indian and Arabian literature.

The recreational use of cannabis in the West can trace its origins to Le Club des Haschischins,
which met in the Hôtel Pimodan on the Île St Louis in Paris in the 1840s. The club was regularly
visited by leading intellectuals, including Honoré de Balzac, Alexandre Dumas and Charles
Baudelaire. Legal restrictions on cannabis first appeared after the International Opium
Convention met in 1925.

Early investigations using cannabis in any of its numerous local formulations had little
scientific value as the content of different active principles varied widely. Clinical studies were
put on a more secure footing after one of the main ones, Δ1,4-trans-tetrahydrocannabinol,
was isolated in 1964 at the Hebrew University in Jerusalem by Raphael Mechoulam and
Yehiel Gaoni. Following up reports that leukaemia patients were less nauseated if they
smoked marihuana before receiving cytotoxic drugs, Stephen Sallan conducted a double-blind
trial comparing tetrahydrocannabinol against placebo, confirming its antiemetic activity when
given by mouth. Several studies have supported this finding, but others reported a high
incidence of cardiovascular and central side effects. Other effects of smoking cannabis have
been considered as the basis of potential clinical applications. One of these is decreased
lacration on exposure to peeling of onions, which was found on further investigation to be
associated with reduced intraocular pressure. This raised the possibility of tetrahydrocanna-
binol being able to reduce intraocular pressure in patients with glaucoma, which was
subsequently confirmed.

Disappointing results followed investigations into the use of tetrahydrocannabinol as an
anti-epileptic drug. Cannabis extract was tested in epilepsy as long ago as 1838 and other
reports subsequently appeared. A pharmacological basis for these was provided in 1947 when
marihuana extract was shown to protect animals against electroshock-induced convulsions.\textsuperscript{37} Several more recent investigations with tetrahydrocannabinol have confirmed this in animals and man, but this has not yet resulted in the licensing of any cannabinoid for clinical use.\textsuperscript{38}

**PACLITAXEL (TAXOL)**

A screening programme was established in 1960 by the US National Cancer Institute to detect antitumour activity in extracts of plants gathered by the US Department of Agriculture. Four years later, samples collected in the State of Washington were given to the Research Triangle Institute in North Carolina for investigation. These included the bark, twigs, leaves and fruit of the Pacific yew tree, *Taxus brevifolia* Nutt. (Taxaceae), extracts of which had been found by the US Cancer Chemotherapy National Service Center to exhibit *in vitro* cytotoxic activity as measured by inhibition of the growth of KB cells. Utilising both conventional solvent extraction and Craig countercurrent distribution techniques, with monitoring of the activity of extracts by their ability to inhibit Walker WM solid tumour growth, Monroe Wall and his colleagues in 1966 isolated 0.5 g of a compound they named ‘taxol’. It was moderately active against murine L1210, P388 and P1534 leukaemias, Walker 256 carcinosarcoma, sarcoma 180 and Lewis lung carcinoma, and was cytotoxic in the KB cell culture system. This was reported in a paper which also revealed the chemical structure of taxol.\textsuperscript{39} Taxol was synthesised in 1994 by two independent groups from Florida State University and the Scripps Research Institute.\textsuperscript{40,41}

Wall has written of his frustration at failing to interest National Cancer Institute administrators in obtaining large quantities of the bark and wood of *T. brevifolia*.\textsuperscript{42} They were understandably apprehensive about the complex extraction procedure which recovered only minute quantities of taxol from a tree in scarce supply. To make matters worse, the substance was insoluble in water and hence difficult to inject. Years passed before two dramatic reports transformed the situation. Firstly, the National Cancer Institute found that taxol was highly active against the relatively resistant murine B16 melanoma. Secondly, Susan Horwitz and her colleagues at the Albert Einstein Medical Center in New York discovered that taxol inhibited cell division at concentrations that had no effect on the synthesis of nucleic acids or protein.\textsuperscript{43} Horwitz went on to establish that taxol acted by binding with the tubulin protein fibres to which chromosomes attach during the metaphase period of cell division. Unlike other drugs that did this, taxol caused realignment of the microtubules and consequent metaphase arrest at concentrations that had no effect on nucleic acid or protein synthesis. These new findings stimulated renewed interest in taxol, with the National Cancer Institute taking the initiative for organising the collection of sufficient bark and wood for the extraction of enough taxol to permit clinical trials. Bristol–Myers Squibb was now brought in to assist with production of the drug and the term ‘taxol’ was dropped in favour of the approved name of ‘paclitaxel’.

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\end{center}

*paclitaxel (taxol)*
A superior source of the drug was also found by converting a product from the Himalayan yew tree (*Taxus baccata*) into paclitaxel.

Early clinical investigations had encouraging results in ovarian cancer, when paclitaxel sometimes produced complete remissions. It is now approved for use in patients with recurrent ovarian cancer and in patients with metastatic breast cancer, often in combination with cisplatin. There is also evidence of its value in lung and prostate cancer.

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Plant Product Analogue and Compounds Derived from Them

Morphine was not only the first active principle to be extracted from a plant but was also the first to have analogues prepared from it. The only way any analogues could be prepared before its chemical structure was established was by modifying one or more of its functional groups. The first semi-synthetic analogue formed in this manner was made by Henry How in 1853 at the Department of Chemistry in the University of Glasgow. In an attempt to elucidate the relationship between codeine and morphine, he heated morphine with several alkyl iodides in alcoholic solution. He had hoped to convert the morphine into codeine, but instead obtained a novel substance that he correctly identified as a quaternary ammonium salt of morphine. How did not take matters further, but 15 years later Alexander Crum Brown, the Professor of Chemistry at the University of Edinburgh, prepared the same quaternary salt together with similar salts of several other alkaloids. He gave these for pharmacological examination to his colleague Thomas Fraser, the Professor of Materia Medica. This resulted in a classic investigation that was one of the first attempts to correlate chemical structure with biological activity. Fraser found that the quaternary ammonium salts of strychnine, brucine, thebaine, codeine, morphine, nicotine, atropine and coniine all exhibited curare-like paralysing activity, despite the pharmacological diversity of their parent alkaloids. This proved that the quaternary ammonium function conferred curariform activity, thereby establishing, for the first time, that certain clusters of atoms could confer specific types of biological activity upon molecules and that the pharmacological properties of natural products could be modified by chemical manipulation. The consequences of this were far-reaching.

A year later, the severely disabled chemist Augustus Matthiessen obtained a post at St Mary’s Hospital Medical School in London, where he conducted research on opium alkaloids. In 1869 he and Charles Alder Wright heated morphine with concentrated hydrochloric acid in a sealed glass tube and obtained a degradation product which they named ‘apomorphia’. Although this was devoid of morphine-like activity, it proved to be a powerful emetic. It was later named ‘apomorphine’ and its structure was elucidated by Robert Pschorr in 1902. Apomorphine has been used in the treatment of impotence and in Parkinson’s disease as it is a potent stimulator of dopamine D_1 and D_2 receptors.

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After the suicide of Matthiessen, Wright continued the research on opiates by preparing morphine and codeine esters, including hydrochloride salts of acetylcodeine, monoacetylmorphine and diamorphine (diacetylmorphine). These were tested on a dog and rabbit by a physician, F.M. Pierce, but his findings were inconclusive because he failed to compare the new compounds with morphine. However, further developments were to take place elsewhere.

The French chemist Edouard Grimaux synthesised ethylmorphine from morphine in 1881. Ralph Stockman, a colleague of Fraser at the University of Edinburgh, and David Dott, a pharmacist, then tested both it and pure diacetylmorphine on frogs and rabbits, observing that ethylmorphine was similar to codeine in its activity. They later reported that while diacetylmorphine had a stronger depressant action than morphine on the spinal cord and respiratory centre, its narcotic action was weaker. Stockman and Dott did not take matters further since their prime interest was on the relationship between chemical structure and biological activity, but the alkaloid manufacturer E. Merck of Darmstadt invited Joseph von Mering to examine 18 esters and ethers of morphine. So far as diacetylmorphine was concerned, he not only confirmed the observations of the Edinburgh researchers, but also tested it on his patients suffering from pulmonary tuberculosis. He reported that it was not only somewhat less potent as a cough suppressant but was also much less potent as an analgesic. However, he found ethylmorphine to be slightly more potent and longer-acting than codeine. Merck then marketed this in January 1898. It was the first semi-synthetic morphine derivative to be made commercially available, being promoted principally as a cough suppressant for use in preference to codeine or other opiates. It is now rarely prescribed.

In 1895, a team of researchers under the direction of Arthur Eichengrün at Friedrich Bayer & Company in Elberfeld, Germany, began preparing a number of esters of phenolic drugs that were known to cause gastric irritation. The expectation was that the formation of an ester would mask the phenolic hydroxyl, which was assumed to be the cause of the irritation. Two years later, Felix Hoffmann synthesised diacetylmorphine. Heinrich Dreser tested it on rabbits and found it both slowed and deepened respiration. This latter aspect encouraged him to examine it in patients as an alternative to codeine for suppressing the agonising coughing of patients with pulmonary tuberculosis while at the same time assisting breathing. Considering it to be a heroic drug with the ability to act as a cough suppressant, yet safer because of its stimulating effects on respiration, he enthusiastically encouraged his company to introduce the drug without delay. Bayer marketed the drug in September 1898 for relief of respiratory disease, using the proprietary name of Heroin. Dreser then published the most detailed pharmacological study yet to have emerged from any pharmaceutical company, though he characteristically failed to acknowledge either the prior communications from Edinburgh and London or the work of his colleagues Eichengrün and Hoffmann. Under the approved name of diamorphine it became popular throughout the world, with many physicians being convinced it was a respiratory stimulant. It was not until 1911 that this opinion was finally shown to be wrong. Higby has described how from increasing popularity as a cough suppressant, diamorphine went on to become widely prescribed as an alternative to morphine for analgesia.
Being unaware that diamorphine is only active after it is rapidly converted in the body to morphine and monoacetylmorphine, Dreser had assumed that Heroin was as non-addictive as the earlier morphine ether derivatives. It is, in reality, one of the most addictive drugs ever introduced into medicine. This was not recognised at first since diamorphine was either administered orally to patients who took it for a short period during an acute respiratory complaint and did not become addicted, or else it was given to patients with chronic tuberculosis and the like in whom treatment was continued and so no withdrawal symptoms occurred. What finally led to the demise of the popularity of diamorphine as a medicinal compound was a report in the *Journal of the American Medical Association* in 1912 warning of its abuse by addicts and calling for legislation to prevent its sale over the counter in drugstores at a price of less than $1 for one hundred 30 mg tablets. The press took up this theme and public opinion began to swing against the ready availability of diamorphine, not only in the United States but around the world. Governments were then compelled to introduce laws controlling the supply of diamorphine and other dangerous drugs.

In 1929, the Rockefeller Foundation, the Committee on Drug Addiction of the US National Research Council, the Public Health Department and the Bureau of Narcotics jointly sponsored a research programme on non-addictive analgesics. For ten years they supported a team of chemists led by Lyndon Small at the University of Virginia and one of pharmacologists led by Nathan Eddy at the University of Michigan. Promising agents were tried on volunteers at the Narcotic Prison Hospital in Lexington, Kentucky. Despite testing about 150 compounds at a cost of millions of dollars, Small and Eddy failed to find a non-addictive agent. The most promising compound to come out of their research was metopon (methyldihydromorphinone), which was about three times as potent as morphine but less likely to cause drowsiness or nausea. Unfortunately, it could not be manufactured at an economical price.

One of the principal clinical problems presented by morphine is its pronounced inhibitory effect on the respiratory centre. In 1942, Chauncey Leake, who had developed vinyl ether as an anaesthetic, suggested to researchers at the Merck Institute in Rahway, New Jersey, that, as some allyl ethers stimulated respiration, an allyl group should be attached to the morphine ring. This was duly done to form nalorphine, the methyl ether of which had previously been synthesised by Julius Pohl in 1915 and reported then as being able to counter the respiratory depression of morphine. This had never been exploited in the clinic. However, Klaus Unna at Merck found that nalorphine was a potent antagonist of morphine and was of considerable value for treating opiate overdosage since it could restore normal respiration within seconds of being injected. Louis Lasagna and Henry Beecher at the Harvard Medical School subsequently discovered that it was also a non-addictive analgesic when given to patients in the absence of morphine. Unfortunately, it caused disturbing hallucinations, ruling out its use as an analgesic.

The establishment of the chemical structure of morphine by Gulland and Robinson in 1922 permitted the synthesis of a wider range of its analogues than had previously been available when it was subjected to simple oxidation, reduction or addition reactions. One of the most
significant developments that now ensued was the synthesis of \( N \)-methylmorphinan, in which the oxygen bridge had been completely removed from the morphine ring system. This structurally simplified analogue of morphine prepared by Rudolph Grewe at Hoffmann-LaRoche in Switzerland had one-fifth the potency of morphine. Grewe next synthesised racemorphan, its hydroxy derivative. Practically all of its analgesic activity was found to reside in the laevorotatory isomer, levorphanol, which was as potent as morphine. It retained addictive liability.

An unanticipated benefit arising from the removal of the oxygen bridge in morphine was an increase in lipophilicity (i.e. the tendency to partition into fat rather than water), as seen in levorphanol. This produced a longer duration of action as the increased lipophilicity ensured extensive distribution throughout the body, which in turn delayed return of the drug to the vascular system prior to its elimination via the kidneys. Additionally, reabsorption of the lipophilic drug from the kidney tubules back into the blood vessels surrounding them also delayed its ultimate excretion. Consequently, levorphanol had a longer elimination half-life than had morphine. This meant less frequent dosing was required, which was especially of value for providing overnight pain relief. The \( N \)-allyl analogue of levorphanol, levallorphan, was subsequently introduced by Hoffmann-LaRoche in 1951. It had more than ten times the potency of nalorphine as a morphine antagonist because its greater lipophilicity favoured entry into the central nervous system.

Cyclorphan was synthesised by Marshall Gates at Rochester University, New York, in 1964. It was a potent morphine antagonist, yet had 20 times the potency of morphine as an analgesic. It could not be used clinically because of psychotomimetic side effects, a problem that persisted with its 14-hydroxy analogue, oxilorphan. However, the cyclobutyl analogue of oxilorphan, synthesised at Bristol Laboratories by Irwin Pachter in 1973, was free of psychotomimetic side effects. It was introduced into the clinic with the approved name of butorphanol. Although about 4 times the potency of morphine, it had limited application as it was only effective when administered parenterally.

**BENZOMORPHANS**

Metazocine, the first benzomorphan, was synthesised in 1957 by Everette May at the National Institutes of Health in Bethesda, Maryland, but was not used in the clinic. The related phenazocine was prepared at the Smith, Kline & French laboratories using May’s methodology. It was highly lipophilic and, unlike morphine, was well absorbed from the
gastrointestinal tract. Tablets could be given for the control of severe pain. Although it had a similar addictive potential to morphine in man, it had the advantage of being about three times as potent an analgesic. It is no longer available in the United Kingdom.

Cyclazocine was patented in 1962 by Sydney Archer of the Sterling–Winthrop Research Institute. It has been used as a morphine antagonist in the treatment of narcotic dependence. Although about 20 times as potent as morphine as an analgesic, it could not be used clinically since it caused severe malaise in patients. The search for an analogue free of this problem resulted in the development of pentazocine by Archer. It was little better than codeine when taken by mouth and had only about one-third of the potency of morphine when injected. Because of its similarity to codeine, its addictive liability was low, but it could induce hallucinations when injected. It is prescribed by mouth for moderate pain.

THEBAINE ANALOGUES

Thebaine has served as a naturally occurring synthetic intermediate rather than as a drug prototype. Compounds were initially synthesised from it without any obvious pharmacological or therapeutic objective, but introduction of a 14-hydroxyl group with appropriate stereochemistry increased the analgesic potency of morphine and codeine analogues. Oxycodone was synthesised in 1916 from thebaine by Martin Freund and Edmund Speyer at the University of Frankfurt. An alternative synthetic route was then developed by Knoll, then the biggest commercial producer of codeine in Germany. Oxycodone is still used in suppositories for patients receiving terminal care. The related oxymorphone, synthesised at the National Institute of Health by Ulrich Weiss in 1955, has ten times the potency of morphine when injected and is an alternative to morphine.
The oxymorphone analogue naloxone was synthesised by Jack Fishman when he was working as a part-time assistant in the private laboratory of Mozes Lewenstein, who was also the head of the narcotics division of Endo Laboratories in New York. This was intended to be a potent analgesic, but on testing at the Endo Laboratories (to whom it was licensed by Lewenstein), naloxone was found to be solely a potent morphine antagonist. A patent was applied for in March 1961, but it was another five years before it was compared with other morphine antagonists and found to be not only the most potent of them all but also of low toxicity and devoid of the tendency to produce the respiratory depression seen with nalorphine. Naloxone was the first pure morphine antagonist and proved invaluable for investigating drug interactions with opiate receptors. It remains the safest antagonist available for the treatment of opiate overdose or poisoning. Naltrexone has similar activity to naloxone, but with a duration of action extending up to three days, and is thus more effective for treating opiate dependence.

Non-addictive analgesics devoid of hallucinatory properties were not available until 1968. Nalbuphine, which was made by Pachter, was three to four times as potent as pentazocine when injected and almost as potent as morphine. It appeared to cause less nausea and vomiting than morphine, hence it was introduced into the clinic for the parenteral treatment of moderate to severe pain. However, when given by mouth it was no more active than codeine.

Taking advantage of the capacity of the conjugated diene in thebaine to undergo the Diels–Alder cyclo-addition reaction, Kenneth Bentley synthesised etorphine in 1963 at the research laboratories in Edinburgh of the alkaloid manufacturer J.F. Macfarlane & Company who were collaborating with the consumer products company Reckitt and Colman to find a replacement for codeine. Etorphine turned out to be about 1000 times more potent than morphine, partly because its higher lipophilicity permitted greater penetration into the central nervous system. The enhanced potency offered no clinical advantage over morphine since it was not accompanied by greater safety, but etorphine was valuable in the immobilisation of large animals with a dart gun. Buprenorphine was synthesised at the same time as etorphine and was later found to be a partial agonist of the mu opiate receptors that was free from significant activity at kappa receptors. It was 100 times as potent as morphine when injected. It also had a longer duration of action than other opiates, but when given by mouth it underwent rapid hepatic metabolism. This was avoided by formulating it for sublingual administration, avoiding unwanted hepatic metabolism. Buprenorphine is widely used for pain relief as it has a lower dependence liability than even codeine, but it is also of value in the treatment of opiate addiction. Thus the original objective of the Reckitt research programme was ultimately achieved.

ATROPINE ANALOGUES

More drugs have been derived directly or indirectly from atropine than from any other drug prototype. The process began after the publication of reports by K. Kraut and Wilhelm
Lossen\(^{36}\) that atropine could be split into tropic acid and a base called tropine. Albert Ladenburg prepared synthetic atropine at the University of Kiel in 1884 by heating these two components in dilute hydrochloric acid.\(^{37}\) Later that year, he reacted tropine with a variety of other aromatic acids to produce a series of physiologically active esters which he called ‘tropines’. Homatropine, the tropeine derived from mandelic acid, proved to act more quickly on the eye than did atropine itself, even though it was not as potent. It also had the advantage of not paralysing the eye muscles for several days, an annoying feature of atropine that prevented patients from focusing in order to read. The manufacturer E. Merck in Darmstadt marketed the new tropeine. It was the first synthetic drug introduced commercially that genuinely improved upon nature.

In 1902, the Bayer Company introduced atropine methonitrate, a quaternary ammonium salt of atropine, as a mydriatic for dilation of the pupil during ophthalmic examination. Because of its highly polar nature it penetrated less readily into the central nervous system than did atropine; hence it was introduced for relieving pyloric spasm in infants. Likewise, ipratropium bromide was introduced by C.H. Boehringer Sohn in 1968 for use by asthmatics, reputedly having fewer side effects than atropine.\(^{38}\)

An important advance arose from work initiated at the Landwirtschaftlichen Hochschule in Berlin and continued at the University of Frankfurt by Julius von Braun.\(^{39}\) He was interested in the effects of transposing functional groups within drug molecules. In the case of atropine, he found that the tropate ester moiety could be moved to a different position on the tropine ring system without loss of mydriatic activity. On further investigation, it became evident that the presence of the intact ring was by no means essential for activity: all that was required was a tertiary amine function situated two or three carbons distant from the tropate ester function. In view of the success achieved in the early years of this century by conducting a similar exercise with the chemically related cocaine molecule to yield novel synthetic local anaesthetics, it is surprising that it took until 1922 before von Braun was able to demonstrate that not only could tropic acid be replaced with retention of mydriatic activity but so, too, could the tropane ring. Once the difficulty in obtaining good yields with the synthetic method had been overcome, the way was clear for a plethora of analogues of atropine to be introduced.

In 1929, the acetyl ester of one of the compounds prepared by von Braun was synthesised by K. Toda and marketed by the Swiss company Hoffmann–LaRoche as a remedy for seasickness under the proprietary name of Navigan\(^{40}\). The acetyl function had been introduced as a protecting group during the synthesis to prevent dialkylation of the tropic acid. It was claimed that the activity was comparable to that of atropine. The next development also came from Hoffmann–LaRoche and was based on the reasoning that, as there was a close structural similarity between atropine and cocaine, it would be worth incorporating into an analogue of the former an amino-alcohol which the company already used in the manufacture of its cocaine analogue, known as ‘dimethocaine’. This led to the introduction of amprotropine in 1933.\(^{41}\) It had a fraction of the spasmolytic activity of atropine in the gut, but, being still less

\[\text{homatropine} \quad \text{atropine methonitrate}\]
potent with regard to antisecretory activity, it had a somewhat diminished tendency to cause a dry mouth when effective antispasmodic doses were administered.

Although amprotropine only attained a modest degree of separation of antisecretory from antispasmodic activity, it was the forerunner of the synthetic selective hormone antagonists such as the beta-blockers and histamine H₂ blockers developed by medicinal chemists in the 1960s and 1970s. Its development encouraged Swiss and German pharmaceutical companies to synthesise a vast range of anticholinergic drugs by combining assorted organic acids with amino-alcohols. Among these were drugs like adiphenine, introduced by Ciba and once popular as an antispasmodic. Cyclopentolate was an alternative to atropine as a cycloplegic agent used during eye examination of children and in iridocyclitis to prevent formation of adhesions through its ability to immobilise the iris and ciliary muscles.

**Pethidine (Meperidine)**

An antispasmodic synthesised in 1930 by Otto Eisleb at the Hoechst laboratories of I.G. Farbenindustrie (formed in 1926 by a merger of eight leading German chemical manufacturers, including Hoechst and Bayer) was pharmacologically examined several years later by Otto Schaumann. To his surprise, it caused the tail of a mouse to flip backwards in a sigmoid shape. Schaumann recognised this as the Straub reaction, characteristic of narcotic analgesics. Further tests confirmed the drug to have about one-tenth of the potency of morphine, but with rapid onset of analgesia and a shorter duration of action. In the mistaken belief that since it bore no chemical resemblance to morphine it would be devoid of addictive properties, it was marketed in Germany for the relief of moderate pain. Eventually, it was found to induce tolerance and habituation. In the United Kingdom, it received the approved name of pethidine, while in the United States it became known as meperidine. It has been of great value in relieving pain during childbirth since it does not depress foetal respiration in anything like the manner of more potent analgesics. It is used as an alternative to morphine if a short duration of analgesia is acceptable.
Propoxyphene is an analogue of pethidine in which the piperidine ring has been opened.\textsuperscript{46} Its dextrorotatory isomer, dextropropoxyphene, was found to have less than half the analgesic potency of codeine and has been incorporated in analgesic products.

**Methadone**

Analogues of pethidine developed by I.G. Farbenindustrie just before the Second World War included a series of compounds prepared by Max Bockmühl and Gustav Ehrhart. One of these had strong analgesic activity and was given the name Amidon\textsuperscript{1}. It was not used clinically at that time, probably because of side effects from the high doses being used.\textsuperscript{47}

When the US State Department investigated the wartime activities of the German chemical industry at the end of the war, reports about Amidon\textsuperscript{1} were examined. Information was passed to the Committee on Drug Addiction and Narcotics of the National Research Council, resulting in details being published by Eddy in 1947.\textsuperscript{48} Amidon\textsuperscript{1} (approved name methadone) was found to be an alternative to morphine in most types of pain, causing less sedation and respiratory depression. It was still addictive. Slow metabolic deactivation made it longer-acting, a feature that made it useful as a morphine substitute for addicts.

**Haloperidol**

An analogue of methadone called dextromoramide was one of the early drugs developed in the mid-1950s by Paul Janssen for his newly established Belgian company then known as NV Research Laboratorium Dr C Janssen but later to be called Janssen Pharmaceutica after it merged with Johnson & Johnson in 1961.\textsuperscript{49} Janssen was a chemistry graduate who went on to qualify in both medicine and pharmacology. He became one of the most prolific drug discoverers of the twentieth century. His dextromoramide proved to be a potent addictive analgesic that was active by mouth, with a rapid onset of analgesic action and a brief duration of action. It has been used to supplement the action of other analgesics at times when intense pain is experienced. Following this success, Janssen turned his attention to pethidine and discovered a straightforward method of replacing the methyl group attached to its nitrogen atom. Compound R951 proved to be a potent analgesic, so he lengthened the chain to a butyrophenone in R1187, which turned out to be an analgesic.
It was noted that the mice injected with R1187 became progressively calm and sedated after initially exhibiting typical pethidine-induced excitement, mydriasis and insensitivity to noxious stimuli. The resemblance of the sedation to that produced by the newly introduced tranquilliser chlorpromazine encouraged Janssen to synthesise analogues of R1187 in the hope of finding a tranquilliser devoid of analgesic activity. This was achieved by replacing the ester function with a hydroxyl group. The next step was to prepare literally hundreds of analogues with different substituents in the benzene rings, as this was already known to enhance potency in similar compounds. From this effort haloperidol emerged, in 1958, as the most potent tranquilliser yet to have been discovered.\(^{50}\) It was 50–100 times as potent as chlorpromazine, with fewer side effects. It remains in use as an antipsychotic drug despite its tendency to produce extrapyramidal side effects such as tremor.

Another of the pethidine variants synthesised by Janssen in the 1950s was diphenoxylate.\(^ {51}\) It lacked analgesic activity despite being well absorbed from the gut. This was due to rapid metabolism in the liver after the drug was absorbed from the gut. Making a virtue out of necessity, Janssen exploited the fact that diphenoxylate retained a morphine-like constipating effect. This meant it could be used in the control of acute diarrhoea, without the risk of causing morphine addiction. Its effect was enhanced by formulating it together with small amounts of atropine to delay its absorption from the gut.

In the decade following the discovery of haloperidol and diphenoxylate, over 5000 similar compounds were synthesised and tested, of which 4000 were made in the Janssen laboratories. A dozen or so of these were introduced clinically and, like haloperidol itself, many of them blocked dopamine receptors. The analogues fentanyl\(^ {52}\) and alfentanil\(^ {53}\) are particularly useful as injectable analgesics because they act within 1–2 minutes. Fentanyl is about 500 times as potent as morphine due to its greater lipophilicity, but the anaesthetic effect is of brief duration because of rapid redistribution from the brain to muscle and fatty tissues. It is used for minor surgical procedures.
While examining the antipsychotic drugs their company had developed, Janssen pharmacologists discovered that some had a greater effect on dopamine receptors in the central chemoreceptor trigger zone that regulated vomiting. This lies outside the blood–brain barrier in the floor of the fourth ventricle of the brain, so a search was made for a dopamine antagonist that would not pass the blood–brain barrier, thereby being free of the extrapyramidal side effects that were associated with drugs of this type. This led to the discovery of domperidone as a strong anti-emetic with minimal central effects.\textsuperscript{54} It was marketed in 1982 for prevention of the nausea and vomiting associated with cancer chemotherapy.

Risperidone was introduced by Janssen\textsuperscript{55} and turned out to be one of the last antipsychotic drugs to be marketed because of concern among pharmaceutical companies over the risk of being taken to court because of side effects from centrally acting drugs. It appears to cause fewer extrapyramidal side effects than earlier agents.

**QUININE ANALOGUES**

The original objective for attempting to synthesise quinine was to overcome its high cost brought about through excessive demand during much of the nineteenth century. Various attempts to prepare synthetic analogues of quinine were made even before its correct structure was established in 1944. The first of these was in London in 1856 when William Perkin tried unsuccessfully to synthesise the alkaloid by oxidising allyltoluidine with potassium dichromate. Instead, he fortuitously obtained a dark sludge, which on examination turned out to be exploitable as a purple dye that he was able to produce on a commercial scale under the name mauveine.\textsuperscript{56} This serendipitous discovery launched the synthetic dyestuffs industry, which was to become the single greatest stimulus for the development of organic chemistry.

**Phenazone**

While working in Adolph von Baeyer’s laboratory at the University of Strassburg in the spring of 1875, Emil Fischer had unintentionally synthesised phenylhydrazine. In the summer of that year, von Baeyer moved to the University of Munich where Fischer joined him. Fischer then initiated his celebrated research into the reactions of phenylhydrazine, which were to result in the discovery of a variety of novel cyclic compounds incorporating nitrogen atoms in their structure. Working under his guidance, his cousin Otto and doctoral student Wilhelm Koenigs synthesised several tetrahydroquinolines in the mistaken belief that quinine was a tetrahydroquinoline. This was a landmark in the history of drug discovery because it was the first deliberate attempt to synthesise a substitute for an alkaloid. When Wilhelm Filehne examined the compounds at the University of Erlangen, he found they exhibited antipyretic activity at doses that did not produce the severe side effects associated with quinine.\textsuperscript{57} One of the tetrahydroquinolines was subsequently marketed in late 1882 under the proprietary name of Kairin\textsuperscript{58} by Farbwerke, vorm. Meister, Lucius und Brüning, a dyestuffs manufacturer based in Höchst am Main near Frankfurt and which was later to adopt the name of the town as its
company name. Kairin® was the first medicinal compound any dyestuff company had ever manufactured. A new era had dawned. However, reports that Kairin® was toxic soon appeared.

Fischer continued his work on the reactions of phenylhydrazine after transferring to the University of Erlangen in 1882, where he asked his assistant Ludwig Knorr to investigate the reaction between phenylhydrazine and acetoacetic ester. Knorr, having expected the product of this reaction to be a tetrahydroquinoline, gave it to Filehne who was testing novel tetrahydroquinolines that might replace Kairin®. Filehne found Knorr’s compound to be devoid of antipyretic activity, but suggested its structure should be modified by attaching a methyl group to the ring nitrogen, as this had previously proved beneficial with compounds related to Kairin®. Knorr then discovered that the N-methylated derivative no longer possessed the acidic properties of its parent compound, yet he failed to realise that this change in properties should not have occurred if it had indeed been a tetrahydroquinoline. The new compound turned out to be an antipyretic that was more palatable than either quinine or salicylic acid. Fischer then magnanimously allowed Knorr to take out a patent in his own name. The patent rights were sold in 1884 to Farbwerke, vorm. Meister, Lucius und Brüning. It was marketed under a proprietary name chosen by Knorr while on his honeymoon in Venice, viz. Antipyrin® (the approved name is phenazone). By the time it reached the market, Knorr had established that it was a pyrazolone derivative.

Phenazone was recommended for use in acute rheumatic fever as early as 1885, when its ability to ease joint pain was specifically commented upon. Two years later, a report of its value in relieving headache appeared in the *British Medical Journal*. Its greatest triumph, however, was in 1889 when Antipyrin® became a household name as an antipyretic after a major influenza epidemic swept through Europe. It then remained the most widely used drug in the world until aspirin began to outsell it in the early years of the twentieth century. If any single product can be said to have established the commercial viability of synthetic drugs, it was phenazone. Today, however, it is no longer used because of the risk of the serious blood condition known as granulocytopenia.

After moving to the Institute of Physiological Chemistry at Strassburg University, Filehne suggested to Friedrich Stolz, the chief chemist at Farbwerke, vorm. Meister, Lucius und Brüning, that certain phenazone analogues should be prepared as potential superior antipyretics. When one of these that had previously been synthesised by Knorr was given to him, Filehne found it to be three times as potent as phenazone. This was marketed in 1896 under the name Pyramidon® (the approved name is amidopyrine). Whether it really possessed any significant advantage over the less-potent phenazone is a moot point, but it remained one of the top selling drugs in Europe until, in 1934, it was incriminated in several cases of granulocytopenia.
One way to minimise the risk of granulocytopenia was to administer smaller amounts by injecting it directly into the circulation, but to make this practical the insolvability of amidopyrine had to be overcome. The Geigy Company in Switzerland tackled this by formulating basic amidopyrine as a soluble complex with an acidic analogue of it in a formulation marketed as Irgapyrine®. When it was found that blood levels of the acidic analogue were much higher than those of the amidopyrine, this analogue was marketed in 1952 as phenylbutazone. As an analogue of amidopyrine, it still caused granulocytopenia as well as another serious blood condition known as aplastic anaemia. Notwithstanding, it retained a significant market share for nearly 30 years until regulatory authorities finally demanded its withdrawal. Some analogues of phenylbutazone are still available, such as azapropazone, although this has restrictions on its use.

COCAINE ANALOGUES

In 1892, Albert Einhorn of the University of Munich proposed a chemical structure for cocaine based on an erroneous one that had been suggested for tropine by Georg Merling at the Technischen Hochschule in Hannover. After taking up an appointment with Chemischen Fabrik vorm. E. Schering in Berlin, Merling synthesised an analogue of cocaine containing only the piperidine ring so that he could then determine whether both rings in the cocaine molecule were essential for anaesthetic activity. This proved to be a potent local anaesthetic and was marketed in 1896 by Schering as the first cocaine substitute, under the proprietary name alpha-Eucaine®.

The sole advantage of alpha-Eucaine® over cocaine was that it was more resistant to hydrolysis, hence injections could readily be sterilised. However, it had to be replaced by the more-soluble beta-Eucaine® after patients complained that it had caused a burning sensation on application to the eye or on injection. This also achieved only a modest degree of clinical acceptability since the problem of irritancy had not been eliminated.

Einhorn now decided to prepare analogues based on the other ring of cocaine, still relying on an incorrect structure for the alkaloid. He synthesised several aromatic compounds containing both the carboxylate ester and benzoyl ester functional groups of cocaine, but was unsuccessful in his attempt to reduce the benzene ring in order to obtain his desired cyclohexane derivatives. Rather than abandon the aromatic compounds he was left with, Einhorn reasoned that as cocaine and some of its active analogues were benzoyl esters, it
would be worth while sending his novel benzoyl esters for testing by his former colleague Robert Heinz, who had moved to the University of Erlangen. Several of the esters turned out to be effective, but activity was also unexpectedly found in the phenolic compounds obtained on removal of their benzoyl function by hydrolysis. This was the first ever indication of useful local anaesthetic activity in synthetic compounds that did not contain a benzoate ester function. Einhorn immediately sent one of his phenolic compounds, orthocaine, to Farbwerke Höchst in 1896. As it was a derivative of phenol, they marketed it as a local anaesthetic with antiseptic activity for application to wounds. Its poor water solubility was turned into a virtue by claiming that this ensured the drug remained on wounds to provide pain relief for many hours.

Because orthocaine tended to form lumps, it was expensive to process. It was replaced the following year by its isomer orthocaine new, a compound prepared by chemists at the University of Heidelberg. This gave fine, smooth-flowing crystals, thereby reducing manufacturing costs.

Einhorn next tried to prepare a water-soluble derivative of orthocaine that would be suitable for injection. As he had already established that it was possible to substitute alkyl chains containing up to five carbon atoms on the amino group of his phenolic compounds, he now examined the effect of introducing chains containing an aliphatic amino group in the hope that this would enable formulation of the resulting compounds as water-soluble hydrochlorides that did not produce highly acidic solutions. This had been the problem with the weakly basic orthocaines. In 1898, Heinz confirmed that one of these amino-compounds was effective as an anaesthetic, although its potency was low. Notwithstanding, it was introduced into the clinic as Nirvanin, principally because it was less toxic than either cocaine or the eucaines. It was soon rendered obsolete because it was still irritant.

In 1902, Ernest Fourneau returned to France after having studied with leading German chemists, including Emil Fischer and Richard Willstätter. He believed that the French should not need to import drugs from Germany as they could easily make their own. He persuaded the brothers Camille, Gaston and Emile Poulenc to establish a pharmaceutical chemistry
research laboratory in their factory at Ivry-sur-Seine, with himself as its director. Within a year he had developed a new local anaesthetic, amylocaine, which Poulenc Frères marketed with the proprietary name of Stovaine®, an amusing bilingual pun on Fourneau’s own name. Like Nirvanin®, it was capable of providing water-soluble salts through the incorporation of an aliphatic amino group. Amylocaine was the first drug to achieve the long sought-after goal of being a safe, non-irritant local anaesthetic that could be injected as a substitute for cocaine. It incorporated all the groups present in the cocaine molecule, except for the piperidine ring, as Fourneau had considered this ring would cause toxicity. After all, Socrates had killed himself by taking hemlock, the active principle of which was the piperidine alkaloid conine!

Einhorn had meanwhile been making esters from both aminobenzoic acids and amino-alcohols for initial screening by Biberfield at the Pharmacological Institute in Breslau. The most promising of these, later to be named procaine, was sent to Heinreich Braun at Leipzig for clinical evaluation. Braun immediately recognised that it was far less irritant than any existing agent, but its action was brief unless high concentrations were injected. Doing this produced as much toxicity as when using cocaine. Only four years before this, however, Braun had improved New York surgeon Leonard Corning’s method of prolonging cocaine anaesthesia by using a tourniquet to prevent the drug from being washed away from the injection site. Instead of applying a tourniquet, Braun had mixed cocaine with an adrenal extract to obtain similar results. Brown now combined Einhorn’s local anaesthetic with the newly introduced adrenaline and overcame the problem of its short duration of action. It was then marketed by Farbwerke Höchst as Novocaine®. The safety, lack of irritancy and overall efficacy of procaine with adrenaline ensured that it dominated the field of local anaesthesia for half a century.

**Procainamide and Its Analogues**

Advances in cardiac surgery during the 1930s led Frederick Mautz of Cleveland, Ohio, to investigate several drugs with a view to finding one that could be applied directly to the heart to prevent arrhythmias during the operative or postoperative period. He felt that disturbance of cardiac rhythm might be prevented by local anaesthetics, especially as procaine had already been shown to have some effect on heart muscle. In his experiments on animals, procaine did indeed prove highly effective and superior to cocaine. Mautz then proposed that if a heart proved exceedingly irritable during surgery, or if the beat became irregular, cautious intrapericardial injection of procaine could correct these disturbances.
It was only after the Second World War that procaine was generally used clinically as an alternative to quinidine, the action of which it resembled. Unfortunately, procaine was rapidly metabolised by esterase enzymes in the blood, severely limiting its clinical application. There was also a high incidence of central side effects. For these reasons, cardiologists welcomed the introduction of procainamide in the 1950s. It was resistant to esterases and had a lower incidence of central nervous system disturbance as it was less lipid-soluble than procaine. Procainamide is still administered intravenously to control ventricular arrhythmias, but is no longer used for chronic administration because of the risk of inducing systemic lupus erythematosus, which manifests itself as a butterfly type of skin rash. This syndrome can progress to cause granulocytopenia if treatment is not withdrawn.

While examining 2-chloroprocainamide as an anti-arrhythmic agent at the Laboratoires Delagrange in France in 1957, Laville and Justin-Besançon discovered that it also had anti-emetic properties when given by mouth. Analogues were synthesised, leading to the introduction of metoclopramide as an anti-emetic. Because the tranquilliser chlorpromazine was also an anti-emetic, several hundred derivatives of metoclopramide were then examined for tranquillisating activity, with sulpiride eventually emerging as a potential antipsychotic agent. Conflicting reports were initially received from French psychiatrists, but it ultimately transpired that sulpiride lacked the sedating properties of drugs such as chlorpromazine and haloperidol, making it a valuable agent in the treatment of schizophrenia. Although available in France for many years, sulpiride was not marketed in the United Kingdom until 1984.

**EPHEDRINE ANALOGUES**

Monopolisation of supplies of *Ephedra vulgaris* in 1927 led to a scarcity of ephedrine and consequent high prices. Gordon Alles, a chemist working for an allergy specialist in Los Angeles, synthesised amphetamine as a cheap substitute for ephedrine. He found it produced a prolonged rise in blood pressure when given by mouth. The drug was then passed to Smith Kline & French Laboratories in Philadelphia, where it had already been examining it as a potential nasal decongestant. The company was aware that it had been synthesised at the University of Berlin in 1887 and examined by George Barger and Henry Dale at the Wellcome Physiological Research Laboratories in London during a major study of sympathomimetic amines in 1910.

Smith Kline & French decided to exploit the volatility of the free base, an oily liquid, by formulating it in a plastic inhaler for insertion into the nostril as a nasal decongestant. This was marketed in 1932 as the Benzedrine Inhaler, a product that achieved considerable commercial success until its withdrawal in the 1960s because the amphetamine was being extracted from the inhaler and being abused. Early clinical experience with amphetamine as an oral decongestant in hay fever had revealed its stimulant properties, leading to its introduction in 1935 for the treatment of the abnormal sleepiness in the rare condition known as narcolepsy. Alles found that the stimulant properties of amphetamine were principally associated with the dextrorotatory stereoisomer, dexamphetamine. Amphetamine also proved to be an anorectic for curbing appetite, but this is now considered an inappropriate use as it causes drug dependence and can induce psychotic episodes. Another controversial application was in the treatment of hyperactivity in children.
Analogues of amphetamine have reflected the diverse actions of the drug. Some were initially intended to be pressor agents for use in shock, while others were developed as stimulants. The latter had enhanced lipophilicity to encourage penetration into the central nervous system. Numerous analogues were introduced to treat obesity, usually being selected on the basis of their claimed relative freedom from stimulant effects, but they are of no benefit. Methylphenidate is used in children and adolescents with severe attention deficit/hyperactivity disorder.76

PHYSOSTIGMINE ANALOGUES

The structure of physostigmine was elucidated in 1925 by Edgar Stedman and George Barger at the University of Edinburgh.77 Utilising the miotic action on the eye as a bioassay procedure, Edgar and Eleanor Stedman went on to prepare several structurally simpler analogues, the most potent being miotine.78 John Aeschlimann of the Roche Research Laboratories in Basle then exploited the stability of dimethyl carbamates, overcoming the extreme sensitivity of physostigmine, a monomethyl carbamate, to hydrolytic cleavage.79 Neostigmine, introduced in 1931, proved superior to miotine in the treatment of the muscular disease myasthenia gravis, a rare form of paralysis in which defective neuromuscular transmission is ameliorated by the anticholinesterase activity of neostigmine. Physostigmine also exhibited this action, but the effects were too brief.

At St Alphege’s Hospital in London in 1934, Mary Walker noted a resemblance between myasthenia gravis and curare poisoning.80 Since physostigmine was an antidote to curare, she tried it on her patients. The results were so striking that, after confirmation by others, they made headlines in the national press in March of the following year. Neostigmine was also used and subsequently found to be superior. It was not without its disadvantages, including unwanted cholinergic actions. Attempts to avoid these resulted in the introduction of pyridostigmine.81

PAPAVERINE ANALOGUES

An efficient synthesis by Carl Mannich at the University of Berlin in 1927 facilitated the preparation of papaverine analogues in which either or both of the two pairs of methoxyl groups could be replaced by methylenedioxy functions.82 Within two years, Otto Wolles of Merck and Company of Darmstadt had obtained a patent for a series of papaverine analogues similar to Mannich’s compounds. Merck claimed that one of these, Eupaverin8 (a name later given to a different benzylisoquinoline alkaloid), was less toxic than papaverine. The Chinoin
Company of Budapest marketed several of the early analogues of papaverine, including ethaverine, which was said to be three times as potent as papaverine with reduced toxicity. Ethaverine and Eupaverin\(^\text{®}\) were widely prescribed as antispasmodics in the 1930s.

The early papaverine analogues proved inferior to the atropine analogues that were introduced as antispasmodics in the 1930s. Open chain analogues were subsequently developed, such as mebeverine and verapamil. The former acts directly on smooth muscle and is still used in a wide variety of gastrointestinal complaints.\(^{83}\) Verapamil was synthesised by Ferdinand Dengel of Knoll AG as a potential coronary muscle dilator for possible use in patients suffering from angina, and was introduced clinically in 1968.\(^{84}\) As it caused cardiac depression, it was passed for further investigation to Albrecht Fleckenstein at the University of Freiburg. He established that it prevented contraction of cardiac muscle as well as that of smooth muscle. Observing that this could be reversed by addition of calcium, Fleckenstein proposed that verapamil was acting as a calcium antagonist. Prenylamine, an analogue of methadone developed by Hoechst AG, was also found to have a similar action. Both compounds interfered with the movement of calcium ions into cardiac cells and so were the first substances to be categorised as calcium antagonists.\(^{85}\) The significance of this became apparent in the 1970s when verapamil was shown to be an anti-arrhythmic agent\(^{86}\) and then to be capable of reducing high blood pressure.\(^{87}\)

**Prazosin**

Pharmaceutical scientists became interested in finding inhibitors of cyclic nucleotide phosphodiesterase enzymes after Earl Sutherland at Western Reserve University in Cleveland, Ohio, had reported in 1962 that caffeine, theophylline and other methylxanthines inhibited the phosphodiesterase that catalysed cyclic adenosine monophosphate (cAMP) hydrolysis.\(^{88}\) Sutherland had identified cyclic AMP in tissues, shown that its formation was catalysed by adenyl cyclase and then elucidated its role as an intracellular second messenger in the
propagation of signals initiated by extracellular hormones. He received the Nobel Prize for Medicine in 1971 for his discoveries. Subsequently, it was found that cyclic guanosine monophosphate (cGMP) also acted as a second messenger. The inactivation of both cyclic nucleotides through hydrolysis of their phosphate ring was catalysed by a large variety of phosphodiesterase (PDE) isoenzymes. Eleven families of PDEs are now known, each produced by different genes. Further subdivisions of isoenzymes exist within each family. The realisation that the level of PDEs varies in different organs opened up the possibility of developing specific inhibitors to control their activity. Although several existing drugs were found to inhibit PDEs and a number of inhibitors were discovered, it was not until the 1990s that any useful drug emerged from the extensive research carried out both in industry and academia. One of the main reasons for this was the difficulty in establishing assay procedures that could identify compounds specifically inhibiting one PDE rather than several.

The Pfizer chemist Hans-Jürgen Hess believed that aminoquinazoline analogues of papaverine would resemble the aminopyrimidine moieties of cAMP and cGMP and so might act as phosphodiesterase inhibitors that act as vasodilators with a direct action on vascular smooth muscle. He and his colleagues developed a synthetic route for the preparation
of 2-amino-4-oxoquinazolines containing methoxyl groups placed appropriately to resemble papaverine. The most active compound was dimethylaminoquinazoline. The oxo group was then replaced with an amino group. This was followed by the synthesis of analogues in which the dimethylamino group was replaced by other amines. The introduction of a substituted piperazine ring led to a compound that did not slow the heart rate, as did other hypotensive drugs then in use. Although it had been designed to inhibit phosphodiesterases, this did not occur unless the drug was given at higher doses than used in the clinic. It was then shown to act as an alpha-adrenoceptor blocker, which caused dilation of both veins and arterioles, resulting in reduction of peripheral resistance in the vascular system and a consequent drop in blood pressure. When first introduced into the clinic under the name prazosin, patients complained of side effects. It was later recognised that if lower doses were used the incidence of side effects declined. Prazosin is now prescribed in combination with other drugs for hypertension. Terazosin and doxazosin are similar to prazosin in both their pharmacological and clinical profiles.

THEOXYLLINE ANALOGUES

Theophylline was difficult to formulate because of its lack of solubility in water. This was partially overcome in 1908 by the introduction of aminophylline, a double compound with ethylenediamine that was manufactured in Dr H. Byke’s chemical factory in Charlottenburg. Other double compounds have been marketed, as well as analogues of theophylline substituted on one of its nitrogen atoms, e.g. pentifylline and its metabolite oxypentifylline, both introduced as vasodilators for peripheral vascular insufficiency.

Dipyridamole

Dipyridamole was one of a series of compounds described as homopurines in a patent granted to the Karl Thomae Company in 1959. Many of the compounds dilated slices of coronary arteries. Detailed reports on the pharmacology of one of the homopurines, dipyramidole, considered it to be a promising coronary dilator since it produced a persistent effect without raising the blood pressure or the workload of the heart. Dipyridamole was subsequently introduced into the clinic in the early 1960s as a coronary vasodilator.
Gustav Born of the MRC Thrombosis Research Group in London discovered that adenosine modified the behaviour of platelet thrombi in experimentally injured arteries. When it was examined at the Radcliffe Infirmary in the hope of developing a treatment for vascular thrombosis, it produced a pronounced fall in blood pressure due to its vasodilatory action. Since an investigation into the mode of action of dipyramidole had previously found that it slowed the rate of disappearance of exogenous adenosine from whole blood, an investigation was conducted to see whether dipyramidole could permit lower doses of adenosine that would not be toxic. In the course of this study, it was unexpectedly discovered that dipyramidole itself was a powerful inhibitor of thrombus formation in injured rabbit arteries. Samples of plasma were then collected from volunteers who had taken dipyridamole and it was found that it prevented platelet clumping when the plasma was stirred. Subsequent clinical studies confirmed the ability of dipyridamole to inhibit thrombus formation in the arterial circulation by reducing platelet adhesiveness. Dipyridamole is now combined with oral anticoagulants to prevent thrombosis in patients with prosthetic heart valves. The vasodilating effect for which it was originally introduced is considered of little value.

Sildenafil

A programme to develop a compound that could lower blood pressure by enhancing the activity of atrial natriuretic peptide (ANP) was initiated in 1985 by Simon Campbell and David Roberts of Pfizer UK in Sandwich, Kent. This particular peptide was of interest since it was a vasodilator that also caused the kidneys to excrete sodium and water, hence elevating its level could possibly reduce blood pressure. As it was known that ANP worked by stimulating guanylate cyclase to increase the synthesis of cGMP, the Pfizer researchers decided to seek an inhibitor for the PDE in kidney tissue that destroyed cGMP. They then isolated the isoenzyme free from contamination with others, enabling progress to be made in testing compounds for inhibitory action. As no potent, selective inhibitors of the PDE were known, Nicholas Terrett and his colleagues sought a lead compound from the literature. They chose zaprinast, an unmarketed drug developed by the UK-based May and Baker division of Rhône–Poulenc. It was a xanthine analogue designed as an anti-allergy drug in the wake of reports about the anti-anaphylactic and anti-inflammatory effects of theophylline and other xanthines, but instead lowered blood pressure in vitro and turned out to be a weak inhibitor of the PDE that was involved in the hydrolysis of cGMP. Before the synthesis of analogues began, a detailed comparison was made between zaprinast and cGMP, the natural substrate for the PDE. The electronic distribution in both compounds was found to be similar, while computer graphics demonstrated that the preferred shape of cGMP could still be retained if there was appropriate replacement of the phosphate ring by another moiety. Consequently, a variety of compounds with variations in the heterocyclic ring system (i.e. the one containing nitrogen atoms) of zaprinast was prepared. The most promising of these was a pyrazolopyrimidinone with ten times the potency of zaprinast as an inhibitor of
the enzyme, which was by now being described as PDE5. Encouraged by this, the researchers focused their attention on the benzene ring, seeking to enhance its similarity to the phosphate ring of cGMP. Two key variations were introduced to increase the similarity to cGMP and thereby enhance fit to the active site of PDE5. Firstly, a 3-methyl group in the pyrazolopyrimidinone ring was enlarged. Secondly, a sulfonamide was introduced on the benzene ring to mimic the cyclic phosphate of cGMP. These changes led to the preparation in 1989 of sildenafil as a PDE5 inhibitor, which was 100 times as potent as zaprinast and highly specific in its action.102,103

By the time sildenafil had been prepared over 1600 compounds had been synthesised and the objective of the Pfizer programme had changed to seeking a vasodilator of coronary vessels for the relief of angina. Despite encouraging results in the laboratory, sildenafil proved disappointing when tested on patients with coronary heart disease. One of several side effects was only revealed when participants in a trial of sildenafil on 30 men in the Welsh town of Merthyr Tydfil in 1992 were questioned about their reluctance to return unused tablets when the trial was stopped. On questioning by the physician in charge, it emerged that they had discovered that the drug was inducing an increase in erectile function. Further investigations not only confirmed this effect in healthy volunteers, but also revealed that it was of benefit to those suffering from erectile dysfunction. An explanation of this was forthcoming, thanks to concurrent advances in understanding the role of nitric oxide (NO) as a signalling molecule in the cardiovascular system which stimulated guanylate cyclase and consequent formation of cGMP. It transpired that on sexual stimulation NO was released from nerves in the penis and thereby raised cGMP levels in the corpus cavernosum. This, in turn, caused smooth muscle relaxation which permitted blood to fill the area and cause an erection.104 Sildenafil was eventually launched by Pfizer under the proprietary name Viagra as a treatment for erectile dysfunction in 1998. Several similar compounds have been introduced since then by other companies.

**DICOUMAROL ANALOGUES**

By 1944, more than 100 hydroxycoumarin analogues of dicoumarol had been synthesised in Paul Link’s laboratory at the University of Wisconsin College of Agriculture, the principal differences between them concerning potency, rate of onset of action and duration. Ethyl biscoumacetate proved superior to dicoumarol because it had a quicker onset of action, but this was offset by a shorter duration.105 As the mode of action of the oral anticoagulants is to interfere with the role of vitamin K in the hepatic biosynthesis of clotting factors, there is a time lag before reserves are depleted. Not only does it take 1–3 days for dicoumarol to exert its action, but after discontinuing treatment its effect may persist for four days or more. Coupled with a degree of unpredictability of response, this led to dicoumarol being superseded by drugs such as warfarin and phenindione.
Warfarin was longer-acting and 5–10 times more potent than dicoumarol, hence it was selected in 1948 as a rat poison from the hydroxycoumarins synthesised in Link’s laboratory. The royalties from its extensive sales were vested in the Wisconsin Alumni Research Foundation, hence its name. Despite the urging of Link, warfarin was not accepted as a clinical anticoagulant until after an attempt by a US army recruit to commit suicide by taking rat poison proved unsuccessful. This indicated that warfarin was safer than other anticoagulants. It was marketed by Endo Laboratories of New York and soon became the most commonly used anticoagulant.

Phenindione was a non-coumarin anticoagulant that was more likely than warfarin to cause hypersensitivity reactions. A report of its anti-inflammatory activity initiated a 15-year-long quest by Pfizer researchers to separate the anti-inflammatory from the anticoagulant activity. Screening of derivatives revealed that the anticoagulant activity (as measured by the prothrombin time) was diminished by large substituents at the meta position on the 2-aryl ring. Control of acidity was critical, since weakly acid compounds with a $pK_a$ value above 5.9 were devoid of anti-inflammatory activity. Despite the recognition of this, all the phenindiones investigated had too brief a plasma half-life to offer prospects of clinical usefulness.

A step forward came with a series of 1,3-dioxoisquinoline-4-carboxanilides, of which the most promising was tesicam, which had an extended plasma half-life but was not considered to have sufficient potency.

The next step involved the preparation of more than 50 benzothiazine carboxamides, the best of which was compound A. It had a long half-life and double the potency of phenylbutazone. The replacement of the benzene ring by a variety of heterocycles enhanced the acidity and increased anti-inflammatory potency. Piroxicam emerged as the most effective
variant.\textsuperscript{109} In humans, it had a half-life of about 45 hours and was about ten times as potent as phenylbutazone.

**TUBOCURARINE CHLORIDE ANALOGUES**

In 1946, Daniel Bovet and his colleagues at the Pasteur Institute in Paris initiated a project to synthesise tubocurarine analogues.\textsuperscript{110} Since the structure of the alkaloid proposed by Harold King had two quinoline rings and two quaternary ammonium functions, Bovet’s group synthesised a series of phenolic ethers containing quaternary ammonium functions.

This led to the synthesis of gallamine in 1947.\textsuperscript{111} Its onset of action was quicker than the 3–5 minutes required for tubocurarine, while recovery was briefer than the 30 minutes or more for the natural alkaloid. As it caused acceleration of the heart, gallamine could not be safely used in patients with kidney or cardiac disease.

**Suxamethonium**

Around the same time that Bovet began his study of tubocurarine analogues in Paris, a similar investigation was being undertaken at Oxford by Richard Barlow and Harold Ing.\textsuperscript{112} Their approach was to establish whether varying the distance between the two quaternary ammonium functions in King’s proposed structure for the alkaloid would increase potency. To this end they examined the simplest possible series of compounds containing two quaternary groups at opposite ends of straight carbon chains, the so-called polymethylene bisonium compounds. It was found that the compound with a 10-carbon chain was three times as potent as tubocurarine when assayed by the rabbit head drop test. By a remarkable coincidence, an identical discovery was made simultaneously by William Paton and Eleanor Zaimis at the National Institute for Medical Research in London.\textsuperscript{113} While investigating the ability of the octamethylene bisonium compound to liberate histamine in a cat, they discovered that the animal was being asphyxiated without exhibiting the obvious signs of respiratory distress such as gasping or convulsions. This suggested that the drug was acting as a neuromuscular blocking agent. Contact was made with Barlow and Ing for agreement on simultaneous publications early in 1948. Paton and Zaimis confirmed that the 10-carbon compound was the most potent member of the bisonium series. They also obtained evidence that it acted by depolarising the muscle end plate so as to prevent it responding to acetylcholine. Unlike tubocurarine and gallamine, it did not behave as a competitive antagonist of acetylcholine. The 10-carbon compound subsequently underwent satisfactory clinical trials and received the approved name of decamethonium.
Decamethonium was marketed for only a few years. Due to its resistance to metabolic deactivation, paralysis was unnecessarily prolonged and could not be reversed with physostigmine. This meant that artificial respiration had to be maintained after completion of surgery until the effects eventually wore off. Seeking a solution to this problem, Bovet examined succinylcholine, a bisonium analogue of acetylcholine. Reid Hunt had studied this compound 40 years earlier, but he failed to observe its neuromuscular blocking activity because his animals had been curarised. Bovet, however, found it to produce very brief neuromuscular blockade; its mode of action was the same as that of decamethonium. It was introduced clinically in 1949, with the approved name of suxamethonium (also known as succinylcholine chloride). With a duration of action of only 5 minutes, it proved ideal for short procedures such as passage of a tracheal tube to assist breathing during general anaesthesia or electroconvulsive shock therapy. Alternatively, it could be administered by intravenous drip for longer operations, with the advantage that paralysis could be terminated by turning off the drip. However, prolonged respiratory paralysis occurred in a small proportion of patients due to abnormally low levels of the pseudo-cholinesterase enzyme responsible for rapid metabolism of the drug. This could not be reversed by physostigmine. Another disadvantage was that the inherent similarity to acetylcholine caused muscle twitching prior to paralysis, which often resulted in postoperative cramp.

**Pancuronium**

John Lewis, Michael Martin-Smith, Thomas Muir and Helen Ross at the University of Glasgow synthesised and examined a series of monoquaternary analogues of several aminosteroids obtained from Christopher Hewett and David Savage of the nearby Organon Laboratories in Newhouse. A compound that incorporated an acetylcholine-like residue in ring A of androstane exhibited one-sixteenth of the potency of tubocurarine. Consideration of the nature of decamethonium, together with a comparison of this monoquaternary steroid with the naturally occurring investigational bisquaternary neuromuscular blocking agent known as malouetine, led Hewett, Savage and Roger Buckett to duplicate the acetylcholine-like moiety at both ends of the steroid to form an analogue of suxamethonium. This led on to the development of pancuronium bromide, the pharmacological properties of which were first reported in 1966.

Due to its more rapid onset of action and relative freedom from effects on blood pressure, pancuronium replaced tubocurarine as the relaxant of choice in major surgery. Its onset of action was still slower than that of suxamethonium, while its duration was in the order of 45
minutes. In patients with liver damage, the duration of action could be prolonged because of slow metabolism of pancuronium. The same occurred in those with renal impairment since the rate of elimination of the drug was reduced. Until the introduction of atracurium and vecuronium (see below), pancuronium was the market leader among surgical muscle relaxants.

At the same time as they synthesised pancuronium, Buckett, Hewett and Savage prepared several closely related compounds. Among these was the monoquaternary analogue of pancuronium. At the time of its synthesis, this had not seemed of sufficient interest to warrant detailed study. However, in 1970, Everett, Lowe and Wilkinson of the Wellcome Research Laboratories discovered that tubocurarine was not a bisquaternary compound as previously believed, but instead was a monoquaternary alkaloid. One of the amino groups was tertiary.

This meant that when Organon submitted more than 100 compounds to Ian Marshall in the Department of Pharmacology at the University of Strathclyde in Glasgow for assessment as neuromuscular blocking agents, the monoquaternary analogue of pancuronium was included among them. When the Organon compounds were subjected by Marshall to pharmacological tests measuring muscle relaxing potency and vagal inhibition, this analogue emerged as having the best selectivity ratio between these properties, and only slightly less potent than pancuronium itself. It was introduced into the clinic with the approved name of vecuronium. It was subsequently found to cause fewer side effects than any other neuromuscular blocking agent.

Atracurium

In the late 1950s, John Stenlake in the Department of Pharmacy at the University of Strathclyde began preparing esters related to suxamethonium in an attempt to find an improved analogue of suxamethonium. A change in the direction of his research occurred in 1964 following the extraction and identification of petaline from *Leontice leontopetalum* L., a Lebanese plant used as a folk medicine to treat epilepsy. Petaline not only had a structure resembling half of the tubocurarine molecule but was also unstable. The facility with which it underwent a rapid Hofmann elimination reaction, normally only seen in quaternary ammonium salts under strongly basic conditions at high temperatures, gave Martin-Smith and Stenlake the idea of synthesising tubocurarine analogues based on petaline, with a view to obtaining a rapidly hydrolysed muscle relaxant that would not persist in the body.
Unfortunately, the decomposition of petaline was too slow at physiological pH for this to be achieved. Stenlake, Roger Waigh and George Dewar then sought to obviate this difficulty by conducting a systematic investigation of isoquinoline esters and ketones in which the electron-withdrawing properties of the carbonyl group in different positions within the molecule were correlated with the ease of Hofmann elimination. As a result of this, they were able to obtain a series of muscle relaxants that at room temperature underwent Hofmann elimination at neutral pH. These compounds, however, lacked adequate potency. A further series provided potent compounds, but produced an unacceptable level of vagal blockade. It was then discovered that incorporation of ester functions in the central chain, coupled with the use of tetrahydropapaverine derived end-groups, abolished all major side effects. Furthermore, when the carbon chain between the esters function was lengthened, muscle relaxant potency was enhanced. The most promising compound thus obtained was atracurium mesylate. On investigation at the Wellcome Research Laboratories, it was found to exhibit a pharmacological profile that met all expectations. It underwent both Hofmann elimination and esterase hydrolysis in vivo and was a potent muscle relaxant. The only problem that remained was the hygroscopic nature of atracurium mesylate. This was overcome by formulating atracurium as its besylate salt.

Atracurium was patented in 1977 by Wellcome after the signing of an agreement whereby royalties would be paid to the University of Strathclyde. It was subjected to its first clinical trials by James Payne at the Royal College of Surgeons of England, who confirmed its freedom from unwanted cardiac effects, as well as the speedy recovery of all patients irrespective of the status of their overall health. This latter feature was a reflection of the chemical degradation through combined Hofmann elimination and esterase hydrolysis rather than solely metabolic breakdown. It ensured a short duration of muscle paralysis, even in patients with hepatic or renal disease. Apart from causing some histamine release, there were few side effects from atracurium. It could be used in a wide variety of situations that were previously considered hazardous. For all these reasons, it became, with vecuronium, one of the most frequently used muscle relaxants in surgery.

Atracurium consisted of a mixture of three groups of geometric isomers, constituting a total of ten stereoisomers. The $R$, cis, $R'$, cis isomer, which accounted for 15% of the isomers, was found to be more potent and slightly longer acting. It had the advantage of being free from the risk of causing cardiovascular complications from the release of histamine and was then introduced as cisatracurium.

### KHELLIN ANALOGUES

In the mid-1950s in Belgium, Camille Beaudet and Bun Hoi Phuc at the laboratories of the Société Belge de l’Azote et des Produits Chimiques du Marly synthesised and screened hundreds of analogues of khellin. Benzalone was then marketed as an antispasmodic, followed by benziodarone, which was introduced in Europe as a coronary dilator for angina. The latter was withdrawn after the UK Committee on Safety of Drugs noted 11 cases of jaundice associated with its use. Analogues of benziodarone were then prepared in which the
phenolic group was masked. This led to the development of amiodarone as a coronary dilator, but concern about corneal deposits, discolouration of skin exposed to sunlight and thyroid disorders also led to its withdrawal, in 1967. Seven years later, amiodarone was discovered to be highly effective in the treatment of a rare type of arrhythmia known as the Wolff–Parkinson–White syndrome.\textsuperscript{125} It acted by prolonging the action potential in the cardiac muscle and conducting system, thus prolonging the refractory period. It also proved valuable in the management of arrhythmias resistant to other drugs and was reintroduced in 1980.

In 1955, chemists at the Benger Laboratories in Cheshire began to synthesise khellin analogues as potential bronchodilators. Compounds were screened for the ability to relieve bronchospasm induced in guinea pigs by histamine or carbachol. Active compounds were further examined in sensitised guinea pigs exposed to aerosols of egg white in order to induce a laboratory model of an asthmatic attack. Roger Altounyan, a physician working with the company, had reservations about the validity of these tests. He took the unprecedented step of evaluating the compounds on himself by inhaling a preparation of guinea pig hair knowing this would induce an asthmatic attack. By 1958, he had found that several carboxylated analogues of khellin were effective, but one in particular had unusual properties. Although it had been reported as having no bronchodilating activity, it somehow protected Altounyan from the effects of guinea pig hair. The compound was only effective if inhaled, but this irritated the lungs. This led the company to abandon the project. Nevertheless, those who had been involved were determined to see it to a successful conclusion, even though only two compounds could be tested on Altounyan each week. They surreptitiously continued with the programme.

A breakthrough came in 1963, when it was found that one of their compounds had been contaminated with highly active material. Brian Lee believed the most likely contaminant was that formed by a side reaction between two chromone molecules to form a bischromone. This was put to the test, progress now being faster due to the takeover of Benger by Fisons and the attitude of the new management being positive. A series of bischromones were then prepared for Altounyan to inhale. In January 1965, he reported that sodium cromoglycate, a bischromone prepared by Colin Fitzmaurice, was ideal as a protective inhalant.\textsuperscript{126} Because the effective dose was somewhat inconvenient to administer, Altounyan and a company engineer...
developed a special device that enabled it to be inhaled as a dry powder liberated from a pierced capsule. The results of the first clinical trial were reported in 1967, with the favourable findings confirmed by subsequent clinical experience throughout the world.127

The new drug was given the approved name of sodium cromoglycate. It was subsequently shown to act by stabilising cell membranes in the lung so as to prevent the allergen-induced release of the substances that would otherwise cause bronchoconstriction in allergic asthma patients. Nedocromil was later found to be more potent than cromoglycate in animals and was effective in some patients who did not respond to cromoglycate.128

PODOPHYLLOTOXIN ANALOGUES

Aware that many plant toxins existed in plants as glycosides, which were more potent drugs than their corresponding aglycones, Sandoz chemists led initially by Arthur Stoll began to search for glycosides in *Podophyllum emodi* and *P. peltatum*, which may have been lost during the extraction of podophyllotoxin. In order to prevent any glycosides being degraded during isolation, precautions were taken to inhibit enzymic activity in the freshly dried rhizomes. This resulted in the isolation of podophyllotoxin glucoside and similar glucosides, which were less toxic than podophyllotoxin, but also less potent.129

The Sandoz researchers next prepared a large series of compounds in which either the glycosidic moiety or the podophyllotoxin residue was modified. After ethers and esters of the glucosides were found to offer no advantage, the crude isolation fraction of podophyllotoxin glucoside was treated with benzaldehyde to form a product designated by Sandoz as SP-G, consisting mainly of podophyllotoxin benzylidene glucoside. This was active as an antimitotic agent in animals at a dose level that did not produce depression of the bone marrow, often the limiting factor in the use of anticancer chemotherapy. SP-G was released in 1963 for experimental investigation in the clinic.130
During an examination of the cytotoxic activity of SP-G, it became apparent that its known components could not account for all the biological activity. Furthermore, SP-G significantly lengthened the lifespan of mice inoculated with L-1210 leukaemia, yet none of its known components could do so. It was concluded that an unidentified, highly potent antileukaemic factor must be present. Careful chromatographic fractionation of SP-G resulted in the isolation of this factor, which was identified in 1964 as 4'-demethylepipodophyllotoxin benzylidene glucoside. The Sandoz team then prepared and examined about 60 analogues of it, concentrating especially on condensation products of it with carbonyl compounds. The condensation product with thiophene-2-aldehyde, teniposide, was synthesised in 1965 and soon emerged as a promising candidate for therapeutic evaluation. Though it was inactive by mouth, teniposide was introduced into the clinic in 1967 on an experimental basis. After it had been shown to be effective in reticulosarcoma, Hodgkin’s disease and bladder carcinoma, teniposide was marketed by Sandoz in some European countries in 1976.

The condensation product between 4'-demethylepipodophyllotoxin glucoside and acetaldehyde was synthesised nine months after teniposide. Known as etoposide, it was only about one-tenth as potent as teniposide when tested for in vitro cytotoxic activity, but it was superior in prolonging the lifespan of mice inoculated with L-1210 leukaemia. Unlike teniposide, it was orally active. When tested in the clinic in 1971, it was found of value in the treatment of non-small-cell lung cancer, testicular cancer and lymphomas. It was also the first drug to be effective against acute monocytic leukaemias. Sandoz licensed both teniposide and etoposide to Bristol–Myers in 1978 as they had expertise in the field of cancer chemotherapy. Five years later, etoposide was approved for use in the United States.

The antitumour action of etoposide, teniposide and other condensed-aldehyde derivatives of 4'-demethylepipodophyllotoxin glucoside is different to that of podophyllotoxin. While at high dose levels they act in the same manner as podophyllotoxin, at normal levels they prevent target cells from entering mitosis. This latter mechanism was responsible for the clinical success of etoposide.
PACLITAXEL ANALOGUES

In 1981, the Centre National de la Recherche Scientifique (CNRS) in Paris signed an agreement with Rhône-Poulenc for the investigation of compounds related to paclitaxel. The research was carried out by Pierre Potier and his colleagues at the Institut de Chimie des Substances Naturelles. Taking into account the extensive studies on taxoid chemistry that had been conducted elsewhere, they prepared derivatives of two inactive precursors of paclitaxel, namely 10-deacetylbaccatin and baccatin. Both were present in the needles and heartwood of the European yew (*Taxus baccata*).

In all, some 40 compounds were synthesised and tested for their activity to inhibit tubulin assembly *in vitro*. The most active compound to emerge from this was an ester of the hydroxyl at position 13, which was made in 1986 and was given the name docetaxel. Further tests in the laboratory showed it to be more cytotoxic than paclitaxel and so it was put on clinical trial by Rhône–Poulenc Rorer. It was licensed in various countries from 1994 onwards for use in advanced or metastatic breast cancer and non-small-cell lung cancer.

CAMPTOTHECIN ANALOGUES

Following the failure of camptothecin in the clinic due to its toxicity and also disappointing results with several analogues, attempts were made to determine the exact nature of its action on tumour cells. This led to the discovery by researchers from Johns Hopkins University collaborating with Smith Kline and French Laboratories that camptothecin acted by inhibiting the enzyme topoisomerase I.
As the mode of action of camptothecin was novel, a renewed effort was made to find a superior analogue, resulting in the preparation of topotecan by William Kingsbury and his Smith Kline colleagues in 1990. It is used when other drugs have failed in the treatment of metastatic ovarian cancer. Topotecan was followed by irinotecan, prepared at the Yakult Institute for Microbiological Research, Tokyo. Irinotecan is a prodrug that is metabolised to form the active metabolite of camptothecin. It is given in combination with fluorouracil in the treatment of metastatic colorectal cancer.

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The Origins of Hormone Therapy

The German physiologist Arnold Berthold was the first to find evidence that a gland could secrete a physiologically active substance directly into the blood rather than through a duct. In 1848, he showed that the shrinking of the comb in castrated roosters did not occur if the excised testes were transplanted into the abdominal cavity. As there was no longer any nervous connection to the testes, he concluded that they were secreting some substance into the bloodstream.1

As with the isolation of active principles from plants, the city of Paris figured prominently in early developments relating to mammalian hormones. The common factor here was the influence of the protégés of François Magendie on the development of experimental physiology. The most illustrious of them was his research assistant Claude Bernard, generally considered to be the founder of experimental medicine. Shortly after becoming Magendie’s successor at the Collège de France in 1855, he reported that his investigations on glycogenesis in the liver had established that an internal secretion is released directly into the blood, thereby helping to maintain the milieu intérieur (internal environment) of the body.2 The following year, Charles-Edouard Brown-Séquard, who was Bernard’s friend and later his successor at the Collège, reported to the Académie des Sciences that removal of both adrenal glands from small animals was rapidly followed by muscular weakness, irregularity of breathing and heart beat, lowering of body temperature, vertigo and convulsions, and finally death within 12 hours of removing the adrenal glands. However, if blood from a healthy animal was intravenously injected, survival was prolonged by several hours.3 Brown-Séquard had clearly been influenced by a book written the year before by Thomas Addison, a physician at Guy’s Hospital in London, entitled On the Constitutional and Local Effects of Disease of the Suprarenal Capsules. This described a condition in which patients presented with bronzed skin and anaemia. After increasing weakness and general illness, these patients died. Post-mortem examination revealed that the suprarenal (i.e. adrenal) glands were diseased. Since then, the condition has been known as Addison’s disease.

There were further developments in Paris in 1856. Though Gabriel Constant Colin, the founder of veterinary physiology, is often remembered for his controversies with Bernard and Pasteur, he also proved that the medulla was a distinct and special part of the adrenal gland by showing that it turned blue when treated with ferric sulfate.4 Edme Vulpian similarly observed that it became green if ferric chloride solution was used, but he went further than Colin by also showing that venous blood leaving the gland turned green, whereas there was no colour reaction with the arterial blood entering it.5 Vulpian suggested that the explanation for this was that the medulla synthesised a substance that was secreted into the circulation. It was to take another 40 years before progress was made towards determining the nature of this internal secretion.

ORGANOTHERAPY

At the age of 72, Brown-Séquard self-administered eight injections of an extract of guinea pig testicles. A month later, in June 1889, he created a sensation by telling the Société de Biologie
that animal testes contained an invigorating principle that might be capable of rejuvenating elderly men. His claim was also published in the *Lancet* and caught the popular imagination throughout the world. Debilitated and senescent patients rushed to visit physicians of renown who, in turn, proclaimed that a new era of organotherapy had dawned. Laboratories were established to exploit this novel type of therapy, offering the public a variety of specially prepared glandular extracts at high prices. It seemed to many that the elixir of life had at last been found.

Brown-Séquard had prepared his extract by grinding either a guinea pig or dog testicle in a small volume of water and then filtering it through paper to obtain a clear solution. A company in Geneva subsequently introduced a proprietary preparation called Séquardine, which was manufactured in exactly the same manner. Since Ernst Laqueur later required more than a ton of bovine testicles in order to isolate enough testosterone for a single course of injections, it seems likely that any invigoration experienced by Brown-Séquard was due to autosuggestion.

Charlatans who sought to enrich themselves through offering organotherapy to gullible clients soon gave organotherapy a bad name, but the publicity was not entirely without benefit. In 1873 at Guy’s Hospital in London, William Gull had concluded that some of his female patients were suffering from a form of adult cretinism. This disease, later to become known as myxoedema, was manifested by various mental and physical changes such as fatigue, extreme sensitivity to cold, dryness of the skin and anaemia. Gull had reported atrophy of the thyroid in his patients, but his suggestion that this might be the cause of the disease was not well received. His views were finally vindicated in 1891 in Newcastle-upon-Tyne after George Redmayne Murray had been asked by his superior to implant sheep thyroid into patients suffering from myxoedema. Murray instead followed in the footsteps of Brown-Séquard by subcutaneously injecting the fluid expressed from a freshly removed sheep thyroid gland into a patient gravely ill with myxoedema. Encouraged by the response, he then made aqueous glycerol extracts from sheep thyroid, following a procedure used to extract pepsin (a digestive enzyme) from the stomach lining of pigs. Despite being desperately ill at the outset, his patient made a complete recovery. She continued to receive regular doses of thyroid extract by mouth and remained in good health for 28 years until her death from other causes. Many more patients benefited from this pioneering work by Murray before the thyroid hormones were isolated. The taint of organotherapy had now been removed from the developing field of endocrinology. Still more importantly, the scene was set for a massive endeavour to understand and exploit physiological and biological processes in order to develop new drugs. No longer would reliance have to be placed on products extracted from plants in order to find therapeutic agents.

### THYROXINE

Eugen Baumann, who worked at the University of Freiburg, was particularly interested in the biochemistry of the thyroid gland because there was a high incidence of endemic goitre in that locality. Goitre is a deformity of the neck due to swelling of the thyroid. F. Bayer & Company supplied Baumann with crude thyroid extract obtained from more than 1000 sheep glands by boiling them in dilute sulfuric acid, cooling and then collecting the flocculent precipitate that was deposited. This was extracted with alcohol by Baumann and purified. When his colleague Paul Kraske suggested that it should be examined for the presence of iodine, Baumann found that there was 2.9% iodine present. He soon became convinced that there was a correlation between the iodine content and potency. By 1896 he had isolated a fraction that contained around 10% iodine. When administered to patients, its beneficial effects were similar to those previously obtained with the whole gland. Believing he might have obtained the active principle, Baumann called it ‘iodothyrin’. F. Bayer & Company then marketed it as
Thyroiodin. The subsequent isolation of still more potent fractions soon made it evident that it had not been pure.

Kraske had made his suggestion about the possible presence of iodine in the extracts after reading of recent successes in Germany when thyroid had been fed to patients with goitre. This reminded him of the former controversial use of iodine for the same condition, a remedy introduced in Geneva in 1820 after Jean François Coindet had found it to be the only ingredient likely to account for any beneficial action of burnt sea-sponge in goitre. This old remedy had been widely employed since the Middle Ages. Coindet treated over 150 patients with iodine, but toxicity due to overdosing became a problem which, coupled with the general ineffectiveness of the therapy in patients with long-standing goitre, brought about its eventual abandonment. Prevost, his townsman, claimed in 1846 that a local dietary deficiency of iodine might be the cause of goitre and cretinism. The obvious way to settle this matter was to carry out an analysis of iodine content in food and water, but suitable analytical techniques did not become available until after the middle of the century. Even then, these lacked refinement and the issue was not settled until after the First World War, when David Marine in Ohio finally confirmed Prevost’s suggestion. This led to the introduction of iodine supplements for inhabitants of goitrogenic locales.

Further developments were slow until Edward Kendall joined Parke, Davis & Company in 1910 and was assigned the task of isolating the thyroid hormone. He subsequently moved to the Mayo Clinic in Rochester, Minnesota, where he isolated material containing 23% iodine, double the highest amount previously recorded. By careful attention to details of the initial hydrolysis of the gland, for which he introduced repeated exposure to alkali instead of dilute sulfuric acid, he obtained crystals of thyroid hormone on Christmas Day 1914. When the process was scaled up, however, Kendall was unable to recover active material. It took 14 months before the cause of this setback was traced to decomposition of the hormone by the galvanised iron vessels in which the hydrolysis of the gland had been conducted. These had to be replaced by enamel vessels. By 1917, Kendall had amassed about 7 g of crystals and was able to start clinical studies. He also put forward a structure for thyroid hormone. Believing it was an oxindole, he coined the name ‘thyroxin’, a term that was later altered to thyroxine (this name has been retained despite the fact that the hormone is not an oxindole). Kendall studiously avoided giving the hormone any name suggestive of the discredited idea that iodine was responsible for thyroid activity. Thyroxine became available commercially shortly after this when it appeared in the catalogue of E.R. Squibb & Sons of Brooklyn, New York. The price was so high that it could only be used for laboratory investigations. This meant that when Charles Harington of University College Hospital in London had doubts concerning the proposed chemical structure, he had to devise a more efficient isolation process that would furnish enough material for his own investigations. Supported by the Rockefeller Foundation, he collaborated with Francis Carr at British Drug Houses, managing in 1924 to increase the yield of hormone twenty-fivefold. This enabled the company to reduce the price to one-tenth of what it had been.

Harington proposed that thyroxine could be formed from two molecules of an amino acid, diiodotyrosine. Collaborating with George Barger at the National Institute for Medical Research, Harington synthesised the proposed condensation product in 1927 and found it to be identical to thyroxine extracted from thyroid gland; both samples were optically inactive. This confirmed that the chemical structure proposed by Harington was correct. However, as this structure possessed a centre of asymmetry, it meant that during the alkaline hydrolysis in the first part of the extraction process, thyroxine had undergone racemisation into a mixture of optical isomers. Harington and Barger therefore separated the isomers and established that the laevorotatory isomer was three times more potent than the dextrotatory one. In 1930, Harington isolated this isomer from the gland in a modified extraction procedure that relied upon enzymatic rather than alkaline hydrolysis.
Thyroxine did not become available at a price that competed with that of dried thyroid gland until Benjamin Hems led a team of chemists at Glaxo Research Laboratories to a synthesis of it in 1949.\textsuperscript{21} Even though eight stages were involved, the overall yield of optically active hormone was 26%.

A more potent hormone than thyroxine was isolated in 1952. Jack Gross and Rosalind Pitt-Rivers detected its presence while carrying out experiments on mice fed with radioactive iodine, which became incorporated into their thyroid glands.\textsuperscript{22} This new hormone was identical to a trace contaminant previously found in some samples of synthetic thyroxine. It contained three iodine atoms instead of the four found in thyroxine. Tests at University College Hospital revealed that the new hormone was about twice as potent as thyroxine, and it was suggested that it was the active species formed in the body from thyroxine. The hormone, which was given the approved name of liothyronine, is also obtained by the Glaxo synthesis.

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George Redmayne Murray's work on thyroid extract for the treatment of myxedema had been carried out in the north of England. It is no coincidence that the development which led to the isolation of the first hormone in a pure state also took place there, in the popular spa town of Harrogate. It was here that George Oliver, a physician, dabbled in physiological experiments using instruments he himself made. Oliver spent his spare time in the autumn of 1893 investigating the action of glycerol extracts of various glands upon the diameter of the radial artery in the wrist, using a new instrument which he had recently designed. He found that when a volunteer (his son!) swallowed an extract of sheep adrenal glands, constriction of the artery could be detected.1 It says much for the sensitivity of his haemodynamometer that he was able to detect this, since around 90% of the active principle present in the extract would have been deactivated as it passed through the gut wall.

Oliver then collaborated with Edward Schäfer at the physiological laboratory of University College in London, with detailed studies being carried out on adrenal extracts. The following March, Oliver and Schäfer told the Physiological Society that water, alcohol or glycerin could extract from the adrenal gland a substance with a powerful action upon the blood vessels, the heart and the skeletal muscles of a variety of animals.2 Within a year of this report appearing in print, an adrenal liquid extract was on sale in Germany for topical application to stop bleeding. There was also a paper from Cracow making the same claims as Oliver and Schäfer.3

An attempt by Oliver and Schäfer to isolate and determine the nature of the substance in their extract was thwarted when a colleague of Schäfer, Benjamin Moore, mistakenly concluded that it was a pyridine or piperidine compound.4 However, Sigmund Fränkel in Vienna correctly deduced that the molecule featured both amine and catechol functions.5

The presence of the catechol accounted for the sensitivity of the active compound to oxidation. In 1897 John Abel isolated the grey sulfate of the active principle in impure form at Johns Hopkins University in Baltimore.6 He went on to obtain its inactive but stable benzoate derivative in an almost pure state, now naming the active compound as 'epinephrine'.7 Otto von Fürth at the University of Strassburg meanwhile developed a crude iron complex of the hormone, which was stable enough to be marketed by Farbwerke Höchst under the name Suprarenin8 for use as a haemostatic agent.8

While continuing with his efforts to purify epinephrine, Abel received a visit in the autumn of 1900 from Jokichi Takamine, a Japanese industrial chemist based in New Jersey, who had established a close link with Parke, Davis & Company. Several weeks before this visit, Takamine had overcome the stumbling block to the final purification of epinephrine.9 This involved using the same procedure adopted by Sertürner a century earlier when isolating morphine from an aqueous extract of opium by addition of ammonia, thus avoiding exposure of the alkaloid to strongly alkaline solutions with which it would form a salt that could not be isolated. The new process worked admirably. Takamine obtained 4g of crystalline base, making this the first hormone ever isolated in a pure state.10 Before publishing his findings in January 1901, Takamine patented his process for isolating the adrenal hormone, arranging for it to be marketed by Parke, Davis & Company with the proprietary name of Adrenalin6. In
the US, the hormone was given the United States Approved Name (USAN) of ‘epinephrine’, but in the United Kingdom and British Commonwealth the term ‘adrenaline’ was introduced as the British Approved Name (BAN). In order to avoid the confusion caused by this type of situation, the World Health Organization introduced a Recommended International Nonproprietary Name (rINN) for all drugs. The use of rINNs is now mandatory in the European Union, the sole exceptions being adrenaline and noradrenaline. These both retain their BAN on grounds of safety, rather than their rINNs of epinephrine and norepinephrine.

Thomas Aldrich, who had worked with Abel, moved from Baltimore to Detroit to join Parke, Davis & Company in 1899. He also isolated small amounts of adrenaline in the summer of 1900, but Takamine held the patent. Abel subsequently pointed out that the published elemental analysis revealed that neither preparation of adrenaline was pure. He was correct, for it was eventually recognised that these products were contaminated with a significant amount of noradrenaline.

Friedrich Stolz, director of chemical research at Farbwerke Höchst, began his attempts to synthesise adrenaline in 1903. At that time, two possible structures were being proposed. Stolz prepared a ketonic precursor of one of these, and sent it and several related compounds to Hans Meyer and Otto Loewi at the University of Marburg for evaluation. They found that these ketonic compounds retained many of the physiological properties of adrenaline, but only to a much lesser degree. The compound most closely resembling adrenaline was given the name ‘adrenalone’ and marketed by Höchst as a nasal decongestant and topical haemostat. Meanwhile, at the University of Leeds, Henry Dakin also synthesised adrenalone as one of a series of adrenaline analogues required for a study of the influence of chemical structure on hormonal activity. He obtained similar pharmacological results. It turned out to be anything but a straightforward matter to convert adrenalone to adrenaline. It took until 1906 before Stolz and Fritz Flächter solved the problems involved in large-scale production, the same year that the correct structure was published.

Synthetic adrenaline turned out to have about half the potency of adrenaline extracted from ox adrenals because natural adrenaline consisted solely of the laevorotatory stereoisomer, while the synthetic material contained equal parts of this and the feebly active dextrorotatory isomer. Flächter solved this problem by his discovery that the acid tartrate salt of the unwanted isomer readily dissolved in methyl alcohol, leaving behind the laevorotatory adrenaline acid tartrate to be filtered off. Its activity was identical to that of natural adrenaline. Höchst replaced the earlier synthetic material with the pure isomer.

The most important use of adrenaline was in the treatment of asthma as an alternative to atropine. In 1900, Solomon Solis-Cohen in Philadelphia injected crude adrenal extract in patients with asthma and hay-fever. Three years later, Bullowa and Kaplan reported their successful use in New York of adrenaline injection in asthma, and this became established as the standard therapy for relief of severe asthmatic attacks. Within a few years a nasal spray containing adrenaline was in use for asthma and also as a decongestant for hay-fever and rhinitis. The first report of adrenaline being administered by an inhaler came from a London medical practitioner in 1929.

Adrenaline was also marketed as a haemostatic to control bleeding in surgery and post-partum haemorrhage. It was even incorporated into suppositories for treatment of haemorrhoids. Intravenous adrenaline was administered in sudden cardiac arrest and was also widely used as a pressor agent to reverse the severe drop in blood pressure associated with
surgical shock. This latter use is now deprecated because peripheral resistance is already high in shock and any further increase is dangerous.

**NORADRENALINE (NOREPINEPHRINE)**

Stölzle and Dakin independently synthesised noradrenaline (the prefix ‘nor’ was an acronym derived from *nitrogen ohne radikal*, indicating the absence of a methyl group). Noradrenaline was not used clinically until after the discovery that it was the principal chemical transmitter mediating electrical impulses in the sympathetic nervous system, rather than adrenaline. The long delay before the role of noradrenaline as a neurotransmitter was established was principally due to the difficulty in separating noradrenaline from adrenaline in sympathin.

![Norepinephrine](image)

Ulf von Euler at the Karolinska Institute in Stockholm proved noradrenaline to be the principal sympathomimetic neurotransmitter in humans in 1949. Sterling Winthrop then marketed the laevorotatory isomer for treating clinical shock.

**TYRAMINE**

Walter Dixon of King’s College in London and Frank Taylor of Cambridge University reported to the Royal Society of Medicine in 1907 that an alcoholic extract of human placenta had adrenaline-like effects when intravenously injected. This extract was examined at King’s by Otto Rosenheim, who established that adrenaline was not present. He then discovered that the pressor agent was only present in material that had undergone bacterial decomposition. When the extract was investigated at the Wellcome Laboratories by George Barger and G.S. Walpole, the active substance was identified as tyramine, an analogue of adrenaline.

![Tyramine](image)

Tyramine was formed from the amino acid tyrosine, which was present when protein decomposed. The Wellcome researchers also isolated tyramine from putrified meat extract. Their colleague Henry Dale confirmed that as a pressor agent it had only a fraction of the potency of adrenaline. However, as its duration of action was much longer than that of adrenaline, it was put to clinical use in the treatment of shock, for which purpose it was synthesised and marketed for some years by Burroughs Wellcome.

**DOPAMINE**

Dopamine was synthesised in 1910 by both Carl Mannich and Barger and Arthur Ewins. Barger and Dale found it to be very much less potent than adrenaline as a sympathomimetic agent.
There was no further interest in dopamine until the 1950s, when it was finally shown to be present in the adrenal medulla and sympathetic nerves. After it had been reported that dopamine might also be present in the brain, Arvid Carlsson and his colleagues at the University of Lund employed a sensitive assay that confirmed that the levels of dopamine in brain tissue were similar to those of noradrenaline. The subsequent discovery of high concentrations in the basal ganglia of animal brains was followed by similar findings in human brain tissue. This was a crucial piece of evidence for Carlsson since he had already discovered that the tranquillising action of reserpine in animals could be overcome by administration of levodopa. As levodopa was the biosynthetic precursor of dopamine, it thus appeared likely that reserpine acted by depleting dopamine reserves in the brain. Carlsson then proposed that dopamine was a neurohormone in its own right, subsequently demonstrating that dopamine was present in central neurones. Much evidence has accrued since then to confirm the validity of Carlsson’s hypothesis.

It is now established that in addition to an agonist action at dopamine receptors, dopamine acts at α- and β-adrenoceptors. This makes dopamine a valuable drug in cardiac failure from infarction or surgery, where inadequate cardiac output reduces the circulating blood volume. One of the major consequences of this can be irreversible renal damage, unless adequate blood supply to the kidneys is restored. Dopamine, injected or infused, immediately produces an increase in the contractility of heart muscle (i.e. a positive inotropic effect), resulting in a rapid increase in cardiac output. This is due to its agonist action at β1-adrenoceptors. As dopamine also stimulates dopamine (DA1) receptors in the kidney, the glomerular filtration rate and renal blood flow increases and protects the kidneys from damage.

LEVODOPA

The Italian physician Torquato Torquati in 1913 found a catecholamine present in the broad bean, *Vicia faba* L.28 The Hoffmann–La Roche biochemist Marcus Guggenheim then acquired some Windsor beans from the garden of Fritz Hoffmann, the founder of his company, and extracted L-dihydroxyphenylalanine.29 This was found to be inactive and so was not used in medicine until the discovery of the role of dopamine as a neurohormone, after which it became known by the approved name of ‘levodopa’.

An enzyme known as ‘levodopa decarboxylase’ was discovered in 1939, which degraded any levodopa present in mammalian tissues, so hindering its detection. Both Peter Holtz in Germany and Hermann Blaschko at Oxford then proposed a multistage biosynthetic pathway for the synthesis of adrenaline from L-tyrosine.30,31 In this, L-tyrosine is converted to levodopa, which decarboxylates to form dopamine. Dopamine is the immediate precursor of noradrenaline, which is converted to adrenaline. Consequently, levodopa can be administered to correct the deficiency of dopamine in Parkinson’s disease. It behaves as a prodrug, undergoing metabolic conversion to dopamine after entering the brain.
The introduction of racemic dihydroxyphenylalanine into therapy took place at the University of Vienna, after Oleh Hornykiewicz had acquired evidence that pointed to depletion of reserves of dopamine in the brains of patients with Parkinson’s disease. Hornykiewicz attempted to alleviate the disease by administering 50–150 mg of dihydroxyphenylalanine intravenously to 20 patients, using this metabolic precursor of dopamine since the neurohormone itself could not cross into the brain from the general circulation.32 His results seemed favourable, as were those reported around the same time by André Barbeau at the University of Montreal, who gave dihydroxyphenylalanine by mouth to six patients.33 However, these findings were disputed by others. It was not until 1967 that the treatment protocols were perfected by George Cotzias and his colleagues at the Medical Research Centre in Brookhaven National Laboratory, who demonstrated that oral doses of up to 16 g each day consistently improved the general clinical condition of more than 50% of patients.34 This improvement only lasted while treatment continued. Because of the expense involved, racemic dihydroxyphenylalanine had been used in the early trials. Since then, levodopa (the optically active isomer that is the metabolic precursor of dopamine) has become a universal treatment for Parkinson’s disease.

HISTAMINE

At the International Physiological Congress in 1907, Henry Dale watched a demonstration of the action of an ergot extract on a uterus removed from a cat.35 He thought that the extract contained a potent substance different from the ergot alkaloids that he had previously encountered because it had an immediate stimulant effect on the uterus. The extract, known as ‘ergotinum dialysatum’, had been prepared by a method that allowed putrefaction to occur. To Dale this was especially relevant as his colleagues at the Wellcome Physiological Research Laboratories in London were at that time investigating the action of tyramine, formed by putrefaction of tissue protein, on the uterus and blood vessels.

When Dale returned to London, he asked George Barger to isolate the putative uterine stimulant. This was duly done, but shortly before Barger completed the final identification of this material,36 Ackerman at the University of Würzburg published an account of the decomposition of histidine, an amino acid, by deliberate putrefaction induced by microorganisms.37 The product formed was identical with the compound isolated by Barger. Because of its formation from histidine, Dale called the uterine stimulant ‘histamine’. It was not a new substance, having being synthesised for purely chemical reasons by Windaus and Vogt three years earlier.38 It was unsuitable for therapeutic use due to its wide-ranging physiological effects, though it later found limited use as a vasodilator in the management of peripheral vascular disease, migraine and Ménière’s disease. Side effects due to the stimulation of histamine H1-receptors included flushing, weakness and headache.

The pharmacological actions of histamine were studied by Dale and Patrick Laidlaw.39 They noted that it caused smooth muscles to contract and lowered blood pressure. They also observed that in various species of animals its effects bore a striking resemblance to what had recently been described as ‘anaphylactic shock’, ensuring that continuing attention would be paid to it. Dale expanded his views on this later.
For many years, it appeared that histamine was only present in various tissue extracts as a result of bacterial action. Then, in 1926, claims were made in the United States and Canada that liver extracts could reduce blood pressure. Charles Best, visiting Dale to work on the crystallisation of insulin, brought a sample of a liver extract that had been used in Toronto. It was examined physiologically by Best and Dale and chemically by Harold Dudley and Thorpe at the National Institute for Medical Research. They isolated substantial amounts of histamine and then repeated the extraction process under conditions that ruled out any possibility of formation of the histamine from histidine by putrefaction. This proved that the vasodilator present in liver was histamine. It now became apparent that this also accounted for the activity of gastric and pituitary extracts previously reported to reduce blood pressure by vasodilation.

In 1927, Thomas Lewis delivered a lecture in which he presented evidence that a substance with properties that resembled those of histamine was released when skin cells were damaged by various causes, including immune reactions. Dale then gave his opinion that the substance was histamine or some combination owing its action to histamine. This triggered intense interest in the role of histamine in the body, culminating in the development of the antihistamines. The release of histamine in the body was also eventually demonstrated.

ACETYLCOLINE

In 1898, Reid Hunt joined Abel’s department at Johns Hopkins Medical School, where he became particularly interested in the nature of the blood pressure lowering principle that remained in adrenal extracts after the adrenaline had been removed. He found that choline was responsible for some of this activity, although it was certainly not the principal hypotensive substance present. Reporting his findings to the American Physiological Society in 1900, Reid Hunt suggested that either a precursor or derivative of choline was likely to be the main active principle. He never succeeded in isolating the choline derivative, but in 1906 he and Rene Taveau demonstrated that acetylcholine, a substance synthesised by Adolf von Baeyer 40 years earlier, was more than 100,000 times as potent a hypotensive agent as choline itself.

Although Hunt subsequently established that several other choline derivatives were also active, he could not exploit this clinically since their effects were of only fleeting duration. Nor could Henry Dale, who examined acetylcholine after Arthur Ewins had isolated it from ergot extracts in 1914. However, the similarity of its effects to those resulting from stimulation of the parasympathetic nervous system led Dale to describe it as a parasympathomimetic agent. In 1921, Otto Loewi demonstrated that repetitive stimulation of the vagus nerve supplying an isolated heart immersed in saline solution caused the release of a chemical that slowed down the rate of a second heart with no nerve supply. Loewi finally isolated this ‘vagusstoff’ in 1926 after discovering that its metabolic destruction, which Dale and Ewins had proposed was due to the action of an esterase enzyme, could be prevented by the alkaloid physostigmine, a drug known to produce cholinergic effects. Loewi thus showed the vagusstoff to be acetylcholine. The Nobel Prize for physiology or medicine was awarded to Dale and Loewi in 1936 for their work on chemotransmission.
5-HYDROXYTRYPTAMINE

A vasoconstrictor in blood serum was isolated in 1948 by Maurice Rapport, Arda Green and Irving Page at the Cleveland Clinic in Ohio.\(^{47}\) Initially it was known as ‘serotonin’. The structure was proposed as 5-hydroxytryptamine (5-HT) by Rapport\(^{48}\) and this was confirmed by its synthesis in 1951.\(^{49}\) 5-HT is stored within the blood platelets and produces vasoconstriction only when these rupture during the first part of the blood clotting process that follows wounding. Vittorio Erspamer in Rome was able to confirm that enteramine, a compound he had extracted from the enterochromaffin cells lining the gastrointestinal tract, was identical to serotonin.\(^{50}\) In fact, it was the enterochromaffin cells in which the highest concentration of 5-HT in the body was to be found.

Ernst Florey was the first to detect the presence of 5-HT in nervous tissue, namely the stellate ganglia of clams.\(^{51}\) This was rapidly followed by Page revealing that 5-HT was present in the brains of mammals.\(^{52}\) At Oxford in 1957 Gaddum established that there were at least two types of 5-HT receptors, describing them as the musculotropic ‘D’ receptors and the neurotropic ‘M’ receptors.\(^{53}\) There has been much progress since then, with radio-labelled ligand-binding studies revealing at least seven families of 5-HT receptors and further subtypes within these.

Once 5-HT had been implicated in both inflammatory processes and as a chemotransmitter in the central nervous system, pharmaceutical companies showed considerable interest in its potential for therapeutic exploitation. They focused their efforts on the development of agonists and antagonists which display a high degree of receptor selectivity. Several major drugs have been developed as a consequence of this approach.

GABA

During the 1960s, the presence of \(\gamma\)-aminobutyric acid, or GABA, in the central nervous system was discovered and its role as an inhibitory neurotransmitter was established.\(^{54}\) It is now accepted that GABA is the main inhibitory neurotransmitter in the brain. There are three families of GABA receptors, with further subtypes within these.

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At the University of Strassburg in 1889, Oskar Minkowski suggested to Josef von Mering that his studies on the role of the pancreas in the metabolism of fat might be advanced by investigation of the effects of surgical removal of the pancreas. After the proposed experiment had been carried out on a dog, Minkowski noticed that the animal was urinating excessively. As this was suggestive of diabetes, he tested the urine for sugar and confirmed its presence. Minkowski then removed the pancreas from several more dogs and all of them rapidly developed diabetes.

In 1900, Leonid Ssobolew in St Petersburg demonstrated that ligation of the pancreatic duct did not cause diabetes even though atrophy of the gland occurred. This suggested that the absence of an internal secretion when the gland was removed was the cause of the diabetes that ensued. Ssobolew also noted that after feeding animals with carbohydrate there was an increase in the number of granules in that part of the pancreas described as the islets of Langerhans. This led him to conclude that the islets were the site at which the gland exerted its effect on carbohydrate metabolism. Six years after this, the German pathologist Julius Cohnheim discovered that attempts to isolate a pancreatic hormone had failed because enzymes in the gland were destroying it. He found the problem could be overcome by deactivating these enzymes by boiling the gland prior to alcoholic extraction. This enabled George Zuelzer, a physician in Berlin, to administer an alcoholic extract that maintained the urinary sugar levels of a pancreatectomised dog under control. Three years later, Zuelzer injected five diabetic patients with a pancreatic extract. He reported an improvement in all cases, but supplies ran out and impurities caused fever. This drew the criticism that elevation of temperature might have lowered sugar output, so he spent a further two years purifying his extracts before Farbwerke Hoechst agreed to produce it as Acomatol®. In 1912 a patent was issued and a contract was signed, but Zuelzer never overcame the difficulties in supplying adequate quantities.

In 1908, Ernest Scott decided that his research for his MSc degree in the department of physiology at the University of Chicago would be on pancreatic secretion. He noted at the outset of his investigation that Cohnheim in 1906 had utilised extracts prepared from pancreas in which the digestive enzymatic activity had first been destroyed by boiling in water prior to alcoholic extraction. In 1910, the Berlin physician Erich Leschke had also referred to this, and he pointed out that the enzyme could possibly destroy the hormone during extraction. Scott therefore resolved to inhibit pancreatic enzyme at the start of his extraction procedures, albeit by using less drastic methods than boiling the gland in water. The first method he tried was based on earlier studies by Minkowski. This involved tying off the glandular ducts some time before removal of the pancreas, thereby allowing the enzyme-producing cells of the gland to atrophy. Scott abandoned this procedure because it was not possible to ensure total atrophy. Among the alternative approaches that Scott then tried was an extraction technique in which the fresh, moist gland was first treated with alcohol. This effectively served to inactivate the digestive enzymes. In this manner, he obtained material that caused a temporary diminution in the amount of sugar excreted by pancreatectomised dogs. This was the first safe and potent
pancreatic extract to have been successfully employed in the treatment of experimentally induced diabetes. Scott did not have an opportunity to pursue the problem after completing his thesis.

Among those who tried to isolate the pancreatic hormone in the 1890s was Nicolas Paulesco at the University of Bucharest, who renewed his former interest in pancreatic extract following the publication of Zuelzer’s results. In 1916, he prepared an aqueous extract of the gland. This proved active when injected into a diabetic dog. Enemy occupation of Bucharest prevented him from taking the investigation further until, in July 1921, he reported his successful isolation of the antidiabetic hormone. He called it ‘pancreine’ and described how it lowered blood sugar levels in normal as well as diabetic dogs when administered intravenously. Only a small quantity of a soluble powder was isolated and it was irritant when injected. Before the large-scale production could be organised, a similar material became commercially available as a result of Canadian endeavours.

INSULIN

After returning from service in the armed forces during the First World War, Frederick Banting practised in London, Ontario, as a surgeon. In October 1920, he read a review article which postulated that a substance secreted by the pancreas might be capable of alleviating diabetes. He came to the conclusion that the failure to isolate this could be due to its destruction by the digestive enzymes in the gland; he was unaware that this same suggestion had been made 10 years earlier. On the advice of colleagues, Banting approached Professor John Macleod at the University of Toronto, who explained that an investigation into this would require several months of full-time work. Macleod told Banting that facilities could be provided in his department. This was a generous offer, particularly in view of the fact that Banting was not proposing any radical new approach to a problem that had defeated the efforts of experts in the field for over two decades. Later, Banting was to understate the importance of what Macleod did for him, both by way of instruction on how to proceed with the investigation and by providing the services of Charles Best, a final-year biochemistry student. Best had been instructed in the latest microchemical method which permitted sugar concentrations to be measured using only fractions of a millilitre of blood. It was application of this new technique that enabled the two Canadians to succeed where others had failed. They began their experimental work in the middle of May 1921 in a small laboratory within the university medical building.

By late July, the degenerated pancreas (caused by ligation of the duct) had been removed from a dog, and on the 30th of the month 4 ml of a ground-up aqueous suspension of it was injected into a pancreatectomised dog. Blood sugar levels quickly dropped from 0.20 to 0.12%, subsequently being held at this level by a second injection. This proof that their extraction technique was satisfactory encouraged Banting and Best who, from early August until November, worked night and day injecting dogs with extracts prepared by a variety of methods. Studies of the stability of the pancreatic hormone were also carried out, revealing that it was sensitive to alkali and to the protein-digesting enzyme trypsin. This raised the suspicion that the hormone was a protein. An improvement in the isolation procedure was obtained when Macleod’s original suggestion to Best was finally acted upon, and Scott’s alcoholic extraction method was employed.

The culmination of their endeavours was reached during the Christmas vacation period, when they injected each other with their extract. Other than a little redness at the injection site, there were no untoward effects. Tests on patients now began, using the same extract. It was given the name ‘insulin’ so as to conform with that suggested for the hormone some 12 years earlier. The first to receive insulin, in January 1922, was 12 year old Leonard Thompson, a dangerously ill diabetic who was being treated at the Toronto General Hospital. He was not
expected to live much longer. Unfortunately, the first injections of insulin caused so much local irritation in the sick boy that treatment had to be stopped.

James Collip had been working in Macleod’s department as a Rockefeller Fellow when he agreed to assist with the purification of insulin. He made the important breakthrough of precipitating insulin from aqueous alcoholic extract of the pancreas by pouring this into several volumes of pure alcohol. This precipitate of insulin was then reconstituted for injection, and was given to Leonard Thompson only a few days after the original injections had been withdrawn. This time the effect of the injections was dramatic. The boy was rapidly restored to excellent health in only a few days. He remained in the best of health for several years, receiving daily injections until his untimely death in a motor cycle accident. When attempts were made to scale-up Collip’s manufacturing process to provide insulin for clinical trials, the yield dwindled and supplies ran out. Tragically, this led to the death of several patients who had been responding well. Facilities to produce insulin had, fortunately, been made available at the Connaught Laboratories, established by the University of Toronto to provide antitoxins and vaccines during the First World War when supplies from Europe were unavailable.

By May 1922, with Best in charge, production was established in the Connaught Laboratories at a satisfactory level, a remarkable achievement when it is realised that there was no previous expertise in this type of production available in Canada. Banting and Best had agreed to apply for patents on the process on the understanding that the University of Toronto would accept and administer these. This it did by setting up an Insulin Committee, which at once put all information it had at the disposal of the Eli Lilly Company of Indianapolis. This public-spirited gesture ensured that mass production of insulin could be rapidly put in hand, thus saving the lives of as many diabetics as possible. Similar arrangements were subsequently made with other responsible agencies throughout the world.

The Lilly group itself consisted of about 100 workers, headed by George Walden. He introduced the technique of isoelectric fractionation, in which advantage was taken of the insolubility of different proteins at critical levels of acidity. Adjustment of acidity allowed insulin to be separated from protein contaminants. Within a year, the Eli Lilly Company was providing enough pure insulin to meet the need of all diabetics in the United States. This established the company as a leading American pharmaceutical manufacturer.

In 1923, a Nobel Prize was awarded to Banting and Macleod. Banting shared his portion of the prize with Best to express his dismay at his colleague being overlooked. Macleod, in response, shared his award with Collip, whom he considered had been largely responsible for turning the experimental findings into a practical reality. Exactly 30 years later at Cambridge, Sanger completed a 10 year investigation which won the 1958 Nobel Prize for chemistry for his elucidation of the chemical structure of bovine insulin. It was the first protein to have its amino acid sequence determined.
Pure, crystalline insulin was prepared in 1926 by John Abel of Johns Hopkins University. It caused less local irritation than amorphous insulin, but turned out to be shorter-acting. In Copenhagen, Hans Hagedorn at the Nordisk Insulin Laboratory assumed that the presence of a protein contaminant had accounted for the longer duration of the earlier products. He searched for an acceptable protein that could be added to crystalline insulin, eventually selecting a small protein known as protamine, obtained from fish sperm. Adding this gave a longer-acting insulin preparation that was introduced in 1936. Two years later, Scott at the Connaught Laboratories found that the duration of action of protamine insulin was no longer than that of ordinary insulin after the removal of zinc, an element he had previously found present in crystalline insulin. This led to the introduction of the more reliable protamine zinc insulin for once-daily injection.

Just after the Second World War, Knud Hallas-Møller of Novo Industrie in Denmark realised that the phosphate buffer used in the preparation of insulins was removing zinc and replaced it with acetate buffer. This led to the marketing of the long-acting Lente range of insulins. These did not require addition of protamine to ensure low solubility for slow dissolution in body fluids.

The discovery by Sanger of minor differences in the amino acid sequences of insulins from oxen, cows and humans stimulated Eli Lilly to use recombinant DNA technology to prepare human insulin from bacteria. A similar product was also made by Novo Industrie by changing one of the amino acids in pig insulin to convert it into human insulin. As a result of these developments, patients now receive human insulin.

**Glucagon**

Shortly after the introduction of insulin, John Murlin at the University of Rochester discovered a second hormone in pancreatic extracts. This exhibited the opposite effect to insulin when injected into laboratory animals, raising their blood sugar levels. It was later given the name ‘glucagon’ and shown to be secreted by the α-cells of the islets of Langerhans, whereas insulin was secreted by the β-cells. Shortly after Sanger had determined the chemical structure of insulin, William Bromer and his colleagues at the Eli Lilly laboratories purified glucagon and elucidated its structure. It was synthesised in 1967.

\[
\text{His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg} \\
\text{Thr-Asn-Met-Leu-Trp-Gln-Val-Phe-Asp-Gln-Ala-Arg}
\]

Glucagon regulates glucose metabolism by promoting the conversion of glycogen in the liver to glucose. It can be injected to counteract hypoglycaemia arising from excessive dosage with insulin or oral hypoglycaemic drugs.

**PITUITARY HORMONES**

The pituitary gland, situated at the base of the brain, was one of several glands from which Oliver and Schäfer prepared glycerine extracts in 1895. They found that pituitary extract could increase blood pressure, although the effect was much less marked than with adrenal extracts. William Howell showed that this pressor activity was only present in extracts prepared from the posterior lobe of the gland. In 1908, Thomas Aldrich established that almost all the activity of the entire pituitary gland could be removed by extraction with 0.1–1.0% aqueous acetic acid. This formed the basis of preparing standardised posterior pituitary extracts that met with pharmacopoeial approval.
While working on the pharmacology of ergot extracts, Henry Dale discovered that posterior pituitary extracts had a powerful action on the smooth muscles of the uterus.\textsuperscript{16} By 1909 such extracts were being used to hasten labour. Once the risk of rupture of the uterus had been recognised, the administration of extracts was generally restricted until after expulsion of the placenta so as to produce prompt contraction of the uterus in order to limit post-partum haemorrhage.

In 1913, von den Velden in Düsseldorf\textsuperscript{17} and Farini in Venice\textsuperscript{18} independently discovered that the principal effect of pituitary extract was to inhibit urine production. This led to its use in the treatment of diabetes insipidus, a disease in which copious amounts of watery urine are voided.

A most important advance came from the research laboratories of Parke, Davis & Company in Detroit in 1928, where Oliver Kamm\textsuperscript{19} and his colleagues extracted posterior pituitary lobes with 0.25% aqueous acetic acid (to maintain pH in the range 3.8–4.4), followed by salting out with ammonium sulfate to obtain a precipitate that was separated by further manipulation into two active hormones. These became known as oxytocin and vasopressin, the latter of which increased blood pressure. Oxytocin found widespread use in obstetric practice.

Further purification of these hormones was achieved at Cornell University Medical College by Vincent du Vigneaud, who had previously worked with Abel on the crystallisation of insulin. Hundreds of thousands of hog and beef glands were used during the course of his investigation, which relied mainly on electrophoretic separation. Progress could only be monitored by testing the purified fractions on animals. During the Second World War du Vigneaud turned to working on penicillin. This introduced him to Craig’s newly developed technique of countercurrent distribution. When he renewed his studies on the pituitary after the war, he combined this technique with starch-column chromatography to purify enough oxytocin for analysis. This showed that it was a peptide (i.e. a small protein), consisting of eight amino acids. At this point it was recognised that the oxytocin could be no more than 50% pure, and another two years passed before a reasonably pure crystalline derivative was obtained in 1953.\textsuperscript{20} Du Vigneaud and his colleagues then determined the structure, confirming it by synthesis.\textsuperscript{21} It was the first peptide hormone to be synthesised. For his persistence over a quarter of a century, du Vigneaud finally had the satisfaction of being awarded the Nobel Prize for Chemistry in 1955 for his work on the posterior pituitary hormones.

\[
\begin{align*}
\text{Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–GlyNH}_2 & \quad \text{oxytocin} \\
\text{Cys–Tyr–Phe–Gln–Asn–Cys–Pro–Arg–GlyNH}_2 & \quad \text{arginine vasopressin}
\end{align*}
\]

Du Vigneaud isolated vasopressin as well as oxytocin in 1953. He established that it was a peptide hormone in which two of the amino acids differed from those in oxytocin, at the third and eighth positions.\textsuperscript{22} Conclusive confirmation of the proposed structure was obtained by its synthesis in 1958.\textsuperscript{23}

There are two types of vasopressin. The hormone in humans, cattle, sheep, horses and chickens has an arginine residue at position 8, hence it is called ‘arginpressin’. That found in pigs has a lysine residue in position 8 and is known as ‘lypressin’. Vasopressin is also the antidiuretic hormone and as such is injected to treat diabetes insipidus arising either from pituitary disease or as a complication following surgery for removal of a pituitary tumour.

**SOMATROPIN (HUMAN GROWTH HORMONE)**

Following Minkowski’s observation, in 1887, that pituitary tumours were associated with the characteristic overgrowth of the hands and feet in patients with acromegaly, evidence accrued to support the belief that a growth hormone was produced by the pituitary. Schäfer reported
in 1908 that feeding rats on pituitary tissue accelerated their growth, while Robertson at Berkeley found that feeding young mice on an alcoholic extract of anterior pituitary lobes not only accelerated growth but even extended it beyond normal limits. Herbert Evans and Joseph Long, at the University of California, accelerated the growth of rats by injecting a saline extract of anterior pituitary lobes. These early experiments have been criticised because of the lack of controlled conditions and failure to take into account the influence of pituitary feedback. However, in 1922 at Berkeley, Philip Smith fed pituitary to hypophysectomised tadpoles (i.e. in which the pituitary had been surgically removed) and thereby restored normal growth. This approach became the basis of modern procedures for determining the activity of pituitary extracts. An additional difficulty was that the early extracts of pituitaries were contaminated with other hormones. Choh Hao Li and Evans eventually isolated pure growth hormone from ox pituitaries in 1944. Later, the term ‘somatotropin’ was introduced to describe the hormone, but early hopes that it would be of value in the treatment of human pituitary dwarfs were soon dashed.

In 1954, Grace Pickford at Yale demonstrated that fish growth hormone was active in fish, but not rats, suggesting that growth hormone might be species-specific. Choh Hao Li then purified both human and monkey growth hormones and found that their chemical structures differed from that of the other growth hormones. Monkey hormone was then shown to be active in monkeys, whereas bovine and porcine varieties were not. Finally, human hormone (prepared independently by Li and by Maurice Raben of Tufts University in Boston) was shown to be effective in patients. Li determined the chemical structure of somatotropin in 1966; this was later revised. It was a globular protein containing 191 amino acid residues.

Growth hormone accounts for about 15% of dried cadaver pituitary. Yet, for many years, supplies of somatotropin from cadavers could not satisfy the demand. Tragically, some batches were belatedly found to be contaminated with a virus that caused several deaths from Creutzfeldt–Jakob encephalopathy. This led to the withdrawal of somatotropin obtained from pooled cadaver pituitaries. Subsequently, genetically modified bacteria were used to produce human growth hormone. This was given the approved name of ‘somatropin’ and replaced somatotropin from cadavers. It is used successfully in the treatment of growth failure in children.

GONADOTROPHIN

When Evans and Long injected an extract of the anterior lobe of the pituitary into rats in 1921, they had been surprised to detect stimulation not only of growth but also of the gonads. Seven years later, Evans reported that an acidic extract had maximal effects on sexual maturation.

Gonadotrophin extracted from cadaver pituitary was used to induce ovulation in infertile women. Until 1985, the gonadotrophins were extracted from cadaver pituitary glands. It was then discovered that these were also contaminated with the virus that causes Creutzfeldt–Jakob disease. Since then, gonadotrophin has been obtained by extraction from urine, being known as chorionic gonadotrophin.

MENOTROPHIN

This follicle-stimulating hormone is a glycoprotein of molecular weight around 67 000 that was obtained pure from sheep anterior pituitaries by Li in 1949. It promoted the development of the ovarian follicle and hence was used in women who, as a consequence of hypopituitarism, were unable to ovulate. It received the approved name of ‘menotrophin’. The hormone extracted from the urine of post-menopausal women differs slightly from the human
pituitary hormone and is known as ‘urofollitrophin’. It has similar uses to follicle-stimulating hormone.

**CORTICOTROPHIN**

In 1926, Philip Smith removed the pituitary gland of rats without causing brain damage. Atrophy of the thyroid and adrenal glands ensued, but this was overcome by daily injections prepared from the anterior pituitary of rats. This encouraged James Collip to introduce an extract for clinical use in 1933 for the treatment of adrenal insufficiency. Further refinement of the extraction procedures led to the isolation in 1943 from sheep and pig pituitaries of an impure peptide that was named corticotrophin.

In 1943, James Murphy and Ernest Sturm at the Rockefeller Institute in New York reported that removal of the adrenals greatly increased the susceptibility of rats to transplanted lymphatic leukaemia. Their findings suggested that adrenal hormones inhibit the development of leukaemia. The following year, they showed that the development of transplanted leukaemia cells in mice was inhibited by both deoxycortone acetate and corticotrophin. F.R. Heilman and Edward Kendall at the Mayo Clinic injected large doses of cortisone into mice bearing transplantated tumours of lymphatic origin and also found that the growth of these were inhibited.

Thomas Dougherty and Abraham White at the Department of Anatomy and Physiological Chemistry of Yale University demonstrated that adrenal stimulation with corticotrophin caused shrinkage of lymph nodes. A second important observation was that adrenal stimulation induced a rapid, but short-lived, diminution in the number of white blood cells. Lloyd Law of the Roscoe B. Jackson Memorial Laboratory at Bar Harbor in Maine and Robert Spiers at the University of Wisconsin-Madison demonstrated in 1947 that naturally occurring leukaemic white cells in mice were also sensitive to adrenal hormones released by injection of corticotrophin.

A team of doctors at the Memorial Hospital in New York headed by Cornelius Rhoads carried out a major clinical trial of corticotrophin. The results were dramatic; patients with chronic leukaemias, advanced Hodgkin's disease (malignancy of lymphatic cells) or sarcomas underwent remission and regained good health as the number of malignant cells declined. Unfortunately, these effects were of temporary duration. Sidney Farber, a pathologist at the Harvard Medical School, reported similar results with acute leukaemia patients at the Children’s Medical Center in Boston. Tragically, the remissions again proved to be only temporary. Nonetheless, this was a major step towards the present situation where most children with acute lymphocytic leukaemia, and a majority of all patients with advanced Hodgkin’s disease, can be cured by combining chemotherapy with radiotherapy. Cortisone replaced corticotrophin, but was in turn superseded by prednisolone, which had fewer side effects. This was combined with the administration of cytotoxic drugs.

The value of corticotrophin in the treatment of malignant diseases and also its use by Philip Hench as an alternative to the scarce cortisone in the treatment of rheumatoid arthritis stimulated further work on the purification of corticotrophin (then known as ACTH, i.e. adrenocorticotrophic hormone).

In 1955, Li and his colleagues at Berkeley established the structure of corticotrophin isolated from sheep as a peptide containing 39 amino acids. Human corticotrophin was isolated in 1963. Eight years later, its structure was determined.

Corticotrophin is no longer used in therapy, the direct application of corticosteroids being preferred when steroid therapy is indicated since the outcome is more predictable. However, it is administered in the diagnosis of adrenocortical insufficiency to determine its effect on hydrocortisone levels in the plasma.
The first 24 amino acids of corticotrophin are identical in all species and retain the full biological activity of the entire molecule. Since the occurrence of hypersensitivity (allergic) reactions after administration of corticotrophin derived from animal pituitaries is attributable to the region of the molecule containing amino acids 25 to 39, a molecule containing the first 24 amino acids was synthesised in 1961. Given the name ‘tetracosactrin’, it had identical properties to corticotrophin, but was less immunogenic. Its main use is in the diagnosis of adrenocortical insufficiency.

Lutropin is a peptide hormone that was isolated in 1940 from sheep pituitaries by Li and from pig pituitaries by Shedlovsky. It is a glycoprotein of molecular weight around 30 000. The amino acid sequence of bovine lutropin hormone (LH) was determined in 1971 and that of human LH in 1978. In the female it stimulates the production of progesterone by the ovaries and, in conjunction with follicle-stimulating hormone (FSH), releases oestrogen from ovarian follicles. It induces ovulation and then facilitates the conversion of the follicular cells into the corpus luteum. In the male, LH stimulates the interstitial cells of the testes to synthesise testosterone. Lutropin is not used in therapy since injection of gonadorelin produces the required rise in LH levels.

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During the period of enthusiasm for organotherapy at the end of the nineteenth century, the Viennese gynaecologist Rudolf Chrobak gave tablets prepared from cows’ ovaries to his patients in an attempt to overcome the undesirable effects that ensued after surgical removal of their ovaries. Chrobak then asked his assistant, Emil Knauer, to investigate the effects of transplantation of ovarian tissue in animals. He began by extirpating the ovaries of rabbits and showing that this led to involution of the uterus and cessation of the oestrus cycles. However, when he reimplanted the ovaries elsewhere in the animals, normal ovarian function was restored. His work inspired others to administer desiccated ovaries or extracts of these to patients in the hope of relieving menopausal disorders. The most potent of the extracts available before the First World War were prepared by Henri Iscovesco in Paris and by Otfried Fellner in Vienna. Both used fat solvents (alcohol, ether and acetone) to obtain extracts that produced sexual changes in castrated animals. The Swiss manufacturer Ciba marketed an ovarian extract in 1913. The techniques used in the preparation of these extracts would have failed to separate the compounds produced by the two distinct components of the ovary, viz. the oestrogens produced in the follicles and progesterone produced in the corpus luteum.

In the recently reprinted 1910 edition of his perennial best seller *Old Age Deferred*, the Czech physician Arnold Lorand cited the use in the preceding two decades of ovarian extracts to relieve menopausal symptoms. Such extracts were introduced when organotherapy was at the height of its popularity and would have been of questionable value. The availability of oestrogens in the 1930s permitted more reliable treatment to be given for the alleviation of menopausal symptoms such as hot flushes and decrease of libido. The case for such therapy was advanced further in 1940 when the American endocrinologist Fuller Albright revealed that menopausal osteoporosis was caused by lack of oestrogens. However, it was not until long after oral contraceptives had become popular that hormone replacement therapy (HRT) began to be routinely used in menopausal women. Recently there have been confusing reports concerning the balance between the risks and benefits of HRT.

**OESTROGENS**

Early attempts to isolate sex hormones therapeutically failed through lack of a cheap, quick assay that could determine the amount of hormone present as the extraction procedures were being refined. Edgar Allen and Edward Doisy of Washington University in St Louis overcame this problem in 1923 by taking advantage of an important discovery made six years before by Charles Stockard and George Papanicolaou at Cornell Medical College. The Cornell researchers had found that changes in the appearance of the cells lining the vaginal wall in rodents closely paralleled the phases of the oestrus cycle. By microscopic examination of cells scraped from the vagina in immature mice or rodents, Allen and Doisy were able to monitor the effects of ovarian extracts. Such was the sensitivity of this simple technique that it afforded an accurate assay for oestrogenic activity. It enabled Doisy to make advances in
isolating the pure ovarian hormone, although he faced major difficulties due to the small amount present in the follicular fluid of the porcine ovaries that he extracted.

In 1927, the Berlin gynaecologists Selman Aschheim and Bernhard Zondek used the Allen and Doisy method of detecting ovarian hormone in an attempt to devise a pregnancy test based on changes in the urinary excretion pattern of female hormone. They found that the amount of hormone in urine increased markedly with the onset of pregnancy. With the publication of the Aschheim–Zondek findings, biochemists realised that an alternative source of female hormone had been found and a race to isolate the hormone ensued.

**Oestrone**

In August 1929, Doisy isolated a crystalline oestrogenic hormone from pregnant mares’ urine by first subjecting the urine to acid hydrolysis (which was later shown to hydrolyse both sulfate esters and glucuronides of the hormone) and then taking advantage of its acidic nature to successively partition the hormone between alkaline solution and ether in order to leave behind impurities in the decanted liquors. His work had been supported by Parke, Davis & Company. Two months later, the isolation of 20 mg of this same hormone was reported by Adolf Butenandt from the laboratory of Adolf Windaus at the University of Göttingen. He had worked on a syrupy extract that the Schering–Kahlbaum Company of Berlin had prepared from nearly 2000 litres of pregnancy urine. Early in the following year, Ernst Laquer and his colleagues at the University of Amsterdam also isolated the hormone. The outcome of these investigations was that Parke, Davis & Company in the United States and Schering–Kahlbaum in Germany were able to provide endocrinologists with oestrone and related hormones. In 1935, it was agreed by a committee of the League of Nations that the hormone should be known as ‘oestrone’.

After J.D. Bernal had proved by means of X-ray crystallography that the structure proposed by Windaus and Heinrich Wieland for the sterol known as ‘ergosterol’ was wrong, Otto Rosenheim and Harold King at University College in London deduced the correct one and then went on to propose that the oestrogens may be related to it. The proof was provided after Girard, in Paris, had developed a special reagent that combined with the carbonyl group in oestrone to form a water-soluble derivative. This enabled large amounts of oestrone to be isolated. At the Research Institute of the Cancer Hospital, London (later the Chester Beatty Research Institute), James Cook and his colleagues used material supplied by Girard to confirm the proposed structures for oestrone and oestriol in 1938.
Oestrone was originally used to treat menopausal symptoms, primary amenorrhoea and hormone deficiency states. It was administered either in an oily injection or by mouth, but was superseded by other oestrogens in the 1950s. Oestrone is not very potent by mouth since it undergoes first-pass metabolism in the liver after absorption from the gut to form oestriol, which is then glucuronidated or sulfated to produce an inactive ester that is excreted in the urine.

**Oestriol**

The isolation of a second oestrogenic hormone from human pregnancy urine was reported in the summer of 1930 by Guy Marrian, a research student in the Department of Physiology at University College London.\(^{16}\) This was later named ‘oestriol’, on account of the three hydroxyl groups in its structure. The structure of oestriol was confirmed by Cook at the same time as oestrone. When administered by injection, it seemed less potent than oestrone, but this is now known to be due to its short duration of action. Oestriol therefore has to be formulated as slow-release implants and transdermal patches.

**Oestradiol**

Erwin Schwenk and Fritz Hildebrandt, colleagues of Butenandt, found that hydrogenation of oestrone formed oestradiol, a new substance that was 8–10 times as potent as oestrone.\(^{17}\) Three years later, in 1935, Doisy showed that it was a natural hormone by extracting 12 mg of it from 4 tons of sows’ ovaries.\(^{18}\)

Oestradiol undergoes first-pass metabolism in the liver to form oestriol, which is then either glucuronidated or sulfated to form an inactive polar ester that is excreted in the urine. This means that an oral dose had to be 5–10 times greater than that given by intramuscular injection.

**PROGESTERONE**

Auguste Prenant, a histologist at the University of Nancy, presented histological evidence to the Société de Biologie in 1898 which indicated that the corpus luteum in the ovary was a gland of internal secretion. Ludwig Fraenkel at the University of Breslau subsequently demonstrated that the corpus luteum played a role in ensuring that the developing embryo attached to the wall of the uterus. The French investigators Paul Bouin and Albert Ansel established that this was achieved by inducing changes in the mucosal lining of the uterus in readiness for pregnancy.\(^{19}\) Twenty years later George Corner and his doctoral student Willard Allen, at the University of Rochester, exploited this observation. By monitoring the changes in the uterine lining, they showed that these were dependent on hormone levels and could form the basis of a reliable bioassay for the presence of hormone in extracts prepared from corpora lutea from sows. The following year they prepared a potent extract that prevented abortion in rabbits after surgical removal of the corpus luteum.\(^{20}\)
In 1934, Adolf Butenandt at the University of Gottingen, Willard Allen and Oskar Wintersteiner at Columbia University, Karl Slotta, Heinrich Ruschig and Erich Fels at the University of Vienna and Max Hartmann and Albert Wettstein at the Ciba laboratories in Basle almost simultaneously announced the isolation of the active principle from the corpus luteum. Its chemical structure was established by Butenandt. Because the hormone was capable of maintaining gestation, it was given the name ‘progesterone’.

MALE HORMONES

The work of Brown-Séquard on testicular extracts may have been controversial, yet it stimulated other French researchers to investigate the internal secretions of the testicles. Among these was Albert Pézard, whose studies began at the Faculté de Médecine in Paris in 1911. He found that the development of the cock comb was dependent upon testicular function, receding on castration but growing after implantation of testicular tissue grafts. Pézard reported his findings to the International Congress of Sex Research held in Berlin in 1926. The following year, Fred Koch and his research student Lemuel McGee, at the Department of Physiology in the University of Chicago, developed a simple assay for male hormone based on the work of Pézard. They demonstrated that bovine testicle extracts prepared using fat solvents could be assayed by their ability to induce growth of the capon’s comb. The availability of this assay was to enable the testicular hormone to be isolated.

After Aschheim and Zondek had shown large amounts of female hormone to be present in pregnancy urine, S. Loewe at Dorpat University confirmed that male hormone was to be found in male urine. Butenandt realised that this would be a suitable source from which to extract the hormone, using the cock comb assay to measure the strength of extracts. In 1930, he isolated 15 mg of pure hormone from 25,000 litres of urine after having removed the phenolic oestrogenic fraction. He named the hormone androsterone and elucidated its structure four years later. It was then synthesised from cholesterol at the Ciba Laboratories in Basle by the Yugoslavian chemist Leopold Ruzicka in 1934. Until evidence to the contrary appeared, it was generally assumed that androsterone was the sole hormone produced in the testicles.

Funded by the Organon Company, Ernst Laqueur led a group of researchers at the University of Amsterdam, which included a Hungarian pharmacologist, John Freud, who had previously worked on the cock comb assay with Pézard in Paris. Freud and Laqueur discovered that extracts of male hormone from urine and from testicular tissue that had been standardised so as to exhibit identical potency in the cock comb assay did not have the identical activity when their effects on castrated rats were compared. While both types of extract induced growth in the rat seminal vesicles, the effect of the urinary extract was markedly less than that of the testicular extract. The possibility that a different male hormone might be present in testicular tissue was then seriously considered and led to isolation of 5 mg of testosterone from nearly 1 ton of bovine testicles in 1935. The paper reporting this was submitted on 27 May 1935. It revealed that the new hormone was 10 times as potent as androsterone in the cock comb test and about 70 times more potent in the castrated rat seminal vesicle test. It concluded that androsterone was a urinary metabolite of testosterone, which was the true male hormone.
The chemical structure of testosterone was elucidated by Ruzicka and Wettstein through its synthesis from cholesterol. They applied for a patent for Ciba on the synthetic process before submitting a paper to *Helvetica Chimica Acta* on 31 August 1935. Butenandt and Hanisch had submitted a paper to *Chemische Berichte* one week earlier. Before these papers appeared in print, the structure of testosterone was revealed by Károly Gyula David, who rushed to publish on behalf of the Amsterdam group. Inadvertently, he omitted the name of his colleague who had actually determined the structure before departing on a holiday, namely Bernard Josephy (later changed to B. J. Brent). No correction of this omission was ever published and the matter may not have come to light were it not for the account presented by Marius Tausk.

Testosterone is administered in hypogonadism and other conditions where there is a deficiency of the hormone, such as that arising from testicular or pituitary disease. As it is rapidly metabolised by the liver after oral dosing, it was for many years formulated as sublingual tablets, but nowadays it is usually administered in the form of patches, intramuscular implants or by depot injection of its esters formulated in oil.

**REFERENCES**

Julius Rogoff and George Stewart of Western Reserve University in Cleveland reported in 1927 that dogs from which the outer cortex of the adrenal gland had been removed could be kept alive by repeated intravenous injections of saline extracts of canine adrenal cortex.\(^1\) Frank Hartman at the University of Buffalo, who similarly prolonged the life of adrenalectomised cats by administering aqueous extracts of adrenal glands from cattle, proposed the name ‘cortin’ for the extracts.\(^2\) These early preparations had only slight and variable activity and hence were unsuitable for clinical application.

In 1930, Hartman and Katherine Brownell developed a potent preparation which was the first successfully used to maintain the life of patients with Addison’s disease.\(^3\) Although comatose when treatment began, one of the first patients to be given their extract was revived and lived eight months until contracting pneumonia.\(^4\) Wilbur Swingle and Joseph Pfiffner at Princeton also prepared potent preparations of cortin by initially extracting with ethanol.\(^5\) The residue obtained on evaporation of the ethanol under reduced pressure was then extracted with benzene, thereby greatly reducing contamination with adrenaline. This procedure permitted complete adrenal glands to be utilised, rather than cortices separated from medullae. The extract was given to patients at the Mayo Clinic and, following a successful outcome, Parke, Davis & Company marketed a product based on this preparation.\(^6\) Further improvements in the extraction procedure were introduced, with Marvin Kuizenga of the Upjohn Company developing what became the standard method of purification. In this, a dried acetone extract was dissolved in water and then fatty contaminants were removed with petroleum ether before final extraction of the aqueous solution with ethylene dichloride. This left behind the adrenaline and phospholipids in the aqueous solution, giving the first cortin preparations totally free of adrenaline.\(^7\)

The improved extraction techniques were based upon developments in assay procedures that became more refined, enabling faster progress to be made. In 1927, Carl and Gerty Cori at the State Institute for the Study of Malignant Disease in Buffalo had demonstrated that adrenalectomy depleted carbohydrate stores.\(^8\) Sidney Britton and Herbert Silvette at the University of Virginia then found that the resulting hypoglycaemia could be reversed by injection of adrenal extract, which came to be described as glucocorticoid activity.\(^9\) This led to the development of assays for the hormone based on its ability to restore blood glucose levels to normal. Another assay procedure originated from the recognition by Emil Baumann and Sarah Kurland at the Montefiore Hospital in New York of serious loss of sodium through renal excretion in patients with Addison’s disease.\(^10\) They had temporarily prolonged the lives of adrenalectomised cats by administering sodium chloride. Robert Loeb at Columbia University subsequently found that restoration of sodium levels to normal in animals could serve as an assay for adrenocortical extracts and indicate their mineralocorticoid activity.\(^11\) Assays introduced by Everse and De Fremery, who tested Reichstein’s extracts,\(^12\) and by Dwight Ingle at the Mayo Clinic in Rochester\(^13\) were based on the profound loss of muscle strength observed in patients with Addison’s disease and in adrenalectomised animals.

The breakthrough came in 1936 when a crystalline compound from the adrenal cortex was isolated by Tadeus Reichstein at the Pharmaceutical Institute in the University of Basel.\(^14\)
Although this lacked cortin-like activity, it did exhibit androgenic activity. When it was discovered to be a steroid, it opened up an entirely new perspective on the field of adrenocortical hormones by raising the possibility that other steroids might be present in the adrenal cortex. Reichstein named this compound ‘adrenosterone’ in recognition of its steroid nature.

The first pure, crystalline compound to be extracted from adrenal tissue and possessing adrenocortical hormonal activity was corticosterone. It was isolated in 1937 by Reichstein and his colleagues, who found that 1 mg of it had the activity of 50–100 g of the whole gland. As it has only weak anti-inflammatory combined with strong mineralocorticoid activity, corticosterone found no therapeutic application.

In 1938, Reichstein isolated deoxycorticosterone from an adrenal extract. Although this was the fifth active compound extracted from adrenals, Reichstein had already synthesised it the previous year in an attempt to establish whether a substituent at the 11-position of the steroid nucleus was required for adrenocortical activity. He prepared it from 3-hydroxy-5-etiocholenic acid, which was readily obtainable by oxidative degradation of cholesterol. The penultimate stage of the synthesis had resulted in the formation of the acetate ester of deoxycorticosterone. Removal of the acetate function in the final synthetic stage resulted in the formation of only half of the amount of deoxycorticosterone that was expected. Although a plentiful supply of deoxycorticosterone was obtained by further improvements in synthetic procedures, much higher yields of its acetate were consistently obtained. Most conveniently, the acetate was to prove ideal for formulation in an oily injection, being broken down in the plasma (through esterase hydrolysis) to release free deoxycorticosterone.

The synthesis of deoxycorticosterone acetate produced sufficient quantities for it to become the first adrenocortical steroid available for clinical use – despite being inactive by mouth because of hepatic metabolism after absorption from the gut. Ciba formulated it as an oily injection in 1939, for use as a mineralocorticoid in patients with Addison’s disease. It was administered in conjunction with adrenocortical extract, this combination being considered superior to the extract on its own.

THE INTRODUCTION OF CORTISONE

In 1941, American intelligence agents picked up a rumour that Germany was purchasing vast quantities of adrenal glands from slaughterhouses in Argentina, purportedly for the
production of extracts that would enable Luftwaffe pilots to withstand the stress of flight at high altitudes! Despite remarkable progress in the isolation and structural elucidation of corticosteroids, insufficient quantities had been obtained for any of them to be used therapeutically, other than deoxycorticosterone acetate. For this reason, Kendall and other leading steroid chemists in the United States were invited by the Office of Scientific Research and Development to collaborate with the pharmaceutical industry in the synthesis of an active corticosteroid for the US Air Force to investigate. The one selected was 11-dehydrocorticosterone, which had been isolated in 1936 by Kendall (as his ‘Compound A’) and found to be active.¹⁷ Kendall contacted Merck & Company in Rahway, New Jersey, and, even though military interest had dried up when it was revealed that the rumours from Argentina were without foundation, the collaboration between Merck and Kendall continued even after the war was over.

The synthesis of an 11-oxygenated corticosteroid presented a formidable challenge since no synthetic precursor containing an oxygen at the 11-position was available. The American researchers had been making slow progress until, in 1943, Reichstein reported his synthesis of 11-dehydrocorticosterone from deoxycholic acid. This required 40 stages to remove both the acidic side chain and the 12-oxygen prior to introducing an 11-oxygen and then the desired side chain at the 17-position, followed by introducing unsaturation in ring A.¹⁸ This synthesis was quite unsuitable for commercial production and its complexity discouraged most researchers from proceeding further. Kendall, to his great credit, was undeterred. His perseverance paid off when, collaborating with Lewis Sarett from Merck, he dramatically reduced the complexity of the Reichstein synthesis, allowing Sarett to prepare 5 g of 11-dehydrocorticosterone for clinical trials.¹⁹ The results of these were disappointing; the compound had no clinical advantage over the much less expensive deoxycorticosterone acetate.

Despite this setback, Merck called a meeting of its clinical consultants. It was agreed that an attempt should be made to synthesise a few grams of Kendall’s ‘Compound E’, 17-hydroxy-11-dehydrocorticosterone (now known as ‘cortisone’), of which Sarett had already obtained 1–2 mg from a 37-step synthesis. Compound E had been isolated in 1936 and found to be active in Ingle’s test on muscular strength.¹⁷

The insertion of a hydroxyl group in the 17-position proved a difficult task, but Sarett eventually managed to prepare 5 g of Compound E in 1948.²⁰ Merck & Company distributed 300 samples of Compound E for clinical trial that same year. They were subsequently approached by a colleague of Kendall, Philip Hench, who was head of the Department of Arthritis at the Mayo Clinic. Seven years before, he had told Kendall that when patients with rheumatoid arthritis contracted jaundice, their painful symptoms were markedly diminished for a time. A similar state of affairs often occurred when women with arthritis became pregnant, their pains returning after childbirth. Believing these and other similar observations to be instances of stress inducing the release of a protective hormone, Hench had suggested to Kendall that once adequate supplies of a corticosteroid hormone became available, it should be tried in rheumatoid arthritis.
Hench was given 1 g of Compound E by Sarett, all that had remained of the 5 g that had been synthesised. On the 21 September 1948, he injected 100 mg into a young woman who was desperately ill with arthritis and whose condition was giving rise to great concern. She showed no signs of improvement until after her third daily injection, when the response was truly dramatic. She awoke the following morning to find herself totally free of pain on moving, something she had not experienced for over five years! After a further week of treatment she was able to walk out of the hospital unaided. An urgent request for more Compound E was then sent to Merck. The company responded magnificently. Improvements to the synthesis allowed the production of 1000 g within only a few weeks. As a result, Hench was able to proceed with a limited trial of the hormone in 13 arthritic patients for six months. Once again, remarkable results were achieved, but only so long as the injections continued. It was evident that Compound E did not cure rheumatoid arthritis. It could only suppress the distressing symptoms, but for patients who did not respond to any other therapy this in itself seemed miraculous. Compound E was now renamed as ‘cortisone’. In 1950, the Nobel Prize in Physiology or Medicine was jointly awarded to Kendall, Reichstein and Hench for their work on it.

The press seized on this dramatic new treatment for arthritis with zeal. Some newspapers hastily described the drug as ‘vitamin E’, thereby creating an undeserved (and persistent) reputation for the vitamin as a miracle drug! To avoid further confusion, Compound E was given the approved name of cortisone. When the success of cortisone therapy became known, there was a worldwide cortisone famine, which the pharmaceutical industry was hard pressed to overcome. To meet the demand, Merck scaled up Sarett’s process and by the end of 1949 were able to offer limited amounts of cortisone acetate to physicians at a price of $200/g. A year later, they had produced 1000 kg and reduced the price to $35/g. Within 5 years, the range of clinical applications for cortisone was to embrace over 50 different conditions, including asthma, allergies and skin diseases. The initial overenthusiasm for the drug had, by then, been tempered somewhat as clinicians experienced at first hand the problems resulting from its mineralocorticoid activity, which seriously upset mineral and fluid balance. Nevertheless, it took many years before it was generally accepted that, because of its side effects, systemic therapy with cortisone and related compounds should be reserved for severely ill patients and those who required hormone replacement therapy in Addison’s disease or after pituitary surgery.

**Hydrocortisone**

Reichstein isolated 17β-hydroxycorticosterone (as ‘Substance M’) in 1937, with Kendall isolating it (as ‘Compound F’) soon afterwards. It was found to possess both glucocorticoid and mineralocorticoid activity, later being shown to be the active form of cortisone in the body and interconvertible with cortisone.

![hydrocortisone](image)

Hydrocortisone became available in amounts adequate for clinical study in 1949 after N.L. Wendler and Max Tishler developed a practical synthesis for Merck & Company. Before
Merck & Company was able to produce it on a commercial scale, G.D. Searle & Company set up a novel process that had been developed by Gregory Pincus and Oscar Hechter at the Worcester Foundation for Experimental Biology. This consisted of hundreds of perfusion cells, each holding cattle adrenal glands immersed in a serum-like solution into which was pumped cheap biochemical precursors, from which the glands synthesised 17\(\beta\)-hydroxycortico-sterone. Searle ignored the costs involved and distributed clinical supplies without charge. The corticosteroid then received the approved name of hydrocortisone. Meanwhile, Upjohn put more than 150 chemists to work on its production. They succeeded by using a mould known as *Rhizopus nigricans* to convert cheaply available progesterone to 11-hydroxyprogesterone which, in turn, was readily made into hydrocortisone.\(^{25}\)

**ALDOSTERONE**

Swiss and American chemists realised that biologically active material remained in the solvent mother liquors after the 30 or so known corticosteroids had been recovered from adrenal extracts. Yet it was James Tait, a newly appointed lecturer in medical physics at the Middlesex Hospital in London, who made the next advance. In 1948, Tait and Sylvia Simpson tested the effect of Allen and Hanbury’s Eucortone\(^{1}\), a brand of adrenal extract, on mineral and water balance. It was known that this was disturbed in patients receiving therapy with this extract. Although Tait and Simpson had no grant to support their study, they did have access to radioisotopes of sodium and potassium. This meant that they could study mineral balance after administering Eucortone\(^{8}\) to animals from which the adrenals had been removed. By applying the recently developed technique of paper chromatography, they were able to separate fractions from Eucortone\(^{8}\) that were biologically active, but it still took them three years to finally obtain a new hormone that had an unprecedented ability to promote salt retention.\(^{26}\) They gave this the name ‘electrocortone’, but were persuaded to rename it as ‘aldosterone’ after establishing its full chemical structure in collaboration with Reichstein and Wettstein.\(^{27}\)

Aldosterone is secreted by the adrenal cortex in response to raised circulating levels of angiotensin II. It acts on the distal tubules of the kidney, stimulating reabsorption of sodium and water. There is an associated loss of potassium. Aldosterone is a major causative factor in certain types of oedema.

**REFERENCES**

In 1930, Raphael Kurzrok and Charles Lieb of the Department of Obstetrics and Gynecology at Columbia University in New York observed that the uteri of women undergoing artificial insemination sometimes contracted violently and on other occasions relaxed.\(^1\) Five years later, Ulf von Euler at the Karolinska Institute in Stockholm detected an acid in extracts of monkey, sheep and goat seminal vesicles that lowered blood pressure and caused smooth muscle to contract.\(^2\) He named it ‘prostaglandin’, but no further work was carried out until one of his students, Sune Bergström, began purifying the extract using a Craig countercurrent extraction apparatus. He was then able to establish that unsaturated fatty acids free of nitrogen were present. After Bergström had obtained a $100,000 grant from the Upjohn Company in the mid-1950s, he and his colleagues began using combined gas chromatographic mass spectrometry analysis to isolate small amounts of several different highly potent prostaglandins and then to elucidate their structures. The prostaglandins were subsequently classified as types A to F according to the pattern of functions in the cyclopentane ring, with each type being given a subscript that indicated the number of unsaturated centres in the side chains. A further refinement was to add the suffix \(\alpha\) or \(\beta\) to indicate the stereochemical configuration of ring substituents.

In 1957, Bergström isolated crystals of alprostadil (prostaglandin E\(_1\)) from sheep prostate glands.\(^3\) Within five years, the structures of alprostadil, dinoprost (prostaglandin F\(_{2\alpha}\)) and dinoprostone (prostaglandin E\(_2\)) had been determined.\(^4\) Dinoprostone is one of the most commonly occurring and most potent of the mammalian prostaglandins, but it and the other prostaglandins of the E series proved to be chemically unstable because of the presence in the cyclopentane ring of a \(\beta\)-hydroxy carbonyl, which readily underwent an acid or base catalysed elimination reaction.

![Structures of alprostadil, dinoprost, and dinoprostone](image)

Phillip Beal and his colleagues at the Upjohn laboratories achieved the first synthesis of a natural prostaglandin in 1965.\(^5\) They synthesised alprostadil four years later.\(^6\) When Elias Corey synthesised dinoprostone at Harvard in 1970, he also succeeded in reducing its cyclic...
carbonyl group to obtain dinoprost. The availability of synthetic prostaglandins allowed clinical investigations to be considered for the first time and the Upjohn Company then supplied prostaglandins free of charge to researchers with the expectation of significant therapeutic advances. However, only three of the sixteen naturally occurring prostaglandins have proved to be of any clinical value.

Hope that prostaglandins could be used in the treatment of peptic ulcer was aroused by a report in 1967 that some of the E series inhibited gastric acid secretion. This was dashed when it was found that they were inactive by mouth and had only a very brief duration of action when injected. This was due primarily to metabolic oxidation of the 15-hydroxyl group in the side chain. Furthermore, like many other pharmacologically active molecules occurring in the body, the prostaglandins of the E series lacked specificity of action and so produced unwanted effects such as vasodilation, which caused facial flushing, headache and hypotension. The vasodilating properties of alprostadil have been exploited to dilate the ductus arteriosus in neonates with congenital heart disease.

Because of its ability to induce contraction of the uterus, intravenous infusion of dinoprostone was found to be an effective way of inducing labour. It is now given by the vaginal route, either as pessaries, tablets or gels. Dinoprostone was also licensed in 1972 for the induction of abortion after the first trimester of pregnancy.

Epoprostenol (prostacyclin) is a prostanoid (i.e. prostaglandin-like substance) that was isolated from microsomes of pig and rabbit aorta in 1976 by John Vane of Burroughs Wellcome. It occurs in the walls of blood vessels, where it produces vasodilation and prevents clotting. Like the prostaglandins it is rapidly metabolised, half of it being destroyed within three minutes of injection.

In 1944, the artificial kidney was invented in Holland, the use of which only became possible as a result of the introduction of pure heparin to stop clotting of the blood. The later development of heart–lung bypass techniques in surgery also depended on the availability of heparin. However, in patients exposed to a high risk of bleeding, the use of heparin in such procedures may be hazardous. In those situations, epoprostenol may serve as an alternative since its effects wear off quickly when it is withdrawn.

REFERENCES


Hormone Analogues

The introduction of structurally related analogues of the alkaloids and other plant products cleared the way for similar developments in the field of hormone analogues. However, it is mainly during the last half century that important therapeutic advances have been made. Among these are several drugs that have saved millions of lives and have made their manufacturers undreamt of profits. The same cannot be said of plant product analogues. In part, this is due to the need for patients to take some hormone-derived drugs on a continuing basis. It is also a reflection of the poisonous nature of the plant prototypes from which the analogues were derived.

ADRENALINE ANALOGUES

The chemical and physiological similarities between tyramine and adrenaline persuaded George Barger and Henry Dale to investigate a variety of amines of similar structure. Many were found to produce physiological effects similar to those observed on stimulation of the sympathetic nervous system. For this reason, Dale described them as 'sympathomimetic amines'. None of these compounds appeared to have any significant clinical advantage over adrenaline.

The only adrenaline analogues used before the First World War were inconsequential, namely adrenalone, deoxyepinephrine and adrenalone. The last of these was prepared by Friedrich Stolz as an intermediate in the synthesis of adrenaline and marketed by Hoechst as a topical haemostatic agent and nasal decongestant. Deoxyepinephrine was synthesised by Frank Pyman. Now obsolete, it was marketed for many years by Burroughs Wellcome as a vasoconstrictor and pressor agent. Although having only about one-tenth the potency of adrenaline, it had a longer duration of action.

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Isoprenaline (Isoproterenol)

The introduction of ephedrine, which had a structural resemblance to adrenaline, stimulated interest in the clinical potential of adrenaline analogues, particularly in Germany. In 1927, the Berlin chemist Helmut Legerlotz synthesised both oxedrine (synephrine) and phenylephrine as analogues of adrenaline in which one hydroxyl had been removed from the catechol ring. The former was licensed to C.H. Boehringer of Ingelheim, Germany, who introduced it in 1930 as a vasoconstrictor, while the latter was marketed by Frederick Stearns & Company of Detroit as a decongestant. It was injected to maintain blood pressure during anaesthesia as it was less likely than adrenaline to reduce the perfusion of blood into vital organs.

Isoetharine was developed by Max Bockmühl, Gustav Erhart and Leonhard Stein at the Höchst laboratories of I.G. Farbenindustrie in 1934. The patent claimed it was a bronchodilator with reduced effects on blood pressure and decreased toxicity compared to adrenaline. Many years later, once the selective action of isoprenaline on beta-adrenoceptors had been recognised, it was realised that isoetharine had similar selectivity. Next, Heribert Konzett at the University of Vienna examined analogues of adrenaline in which the methyl group attached to its nitrogen was replaced with either an ethyl, n-propyl, isopropyl or butyl substituent. As was the case with isoetharine, the isopropyl analogue was the most promising compound, being ten times as potent a bronchodilator as epinephrine when injected but free of its hypertensive effects. Boehringer marketed it in 1941 under the name Aludrin, but little attention was given to it due to the wartime situation. It was not until after the US State Department investigated the research conducted during the war by German companies that the clinical merit of the drug was fully appreciated. It was given the approved name of isoprenaline in 1951 (isoproterenol in the United States). Being a catecholamine, it did not cause insomnia as did the more lipid-soluble ephedrine, which penetrated into the brain. For the next 20 years it was the drug of choice for the relief of asthma attacks. Tragically, its introduction in convenient aerosol form during the 1960s may have led to an estimated 3000 deaths among asthmatic teenagers in the United Kingdom alone. If so, it was probably due to the effects on the heart of accidental overdosing.

In the course of examining drugs on smooth muscle to see if they could relieve menstrual cramps, Raymond Ahlquist of the Medical College of Georgia noticed that the effects of isoprenaline and noradrenaline differed. The latter made smooth muscle contract while isoprenaline caused it to relax. Both activities were shown by adrenaline, but varied with the site of action. Ahlquist recognised a parallel with cholinergic receptors, which had been classified in 1914 by Dale as being either nicotinic or muscarinic on the basis of whether nicotine or muscarine stimulated a response. Ahlquist explained the different actions of adrenaline analogues by introducing the concept of two types of adrenoceptors, namely alpha-receptors and beta-receptors. Noradrenaline was an alpha-receptor agonist whereas isoprenaline was a beta-receptor agonist, while adrenaline was a mixed alpha- and beta-receptor agonist. Ahlquist’s contribution ultimately transformed the understanding of the
action of sympathomimetic amines and cleared the way for major therapeutic developments in this field, yet for many years his claims were universally ignored.

**Orciprenaline**

The success of isoprenaline served to highlight one of its disadvantages, even before its cardiac toxicity became a matter of concern. Because isoprenaline was a catecholamine like adrenaline, it was susceptible to enzymes in the gut wall and throughout the body that rapidly deactivated catecholamines. As a result, isoprenaline was inactive by mouth and too short-acting to be employed in the same manner as ephedrine for the prophylaxis of asthma.

Emil Eidebenz and Maxmilian Depner of Chemische Werke Albert in Wiesbaden-Biebrich applied for a patent in 1943 for adrenaline analogues in which one of the two hydroxyl groups had been moved to an adjacent position on the benzene ring. The patent was finally granted ten years later. The compounds, including the orcinol isomer of adrenaline, had similar activity to adrenaline but were more stable since they were no longer catecholamines. This approach was not exploited clinically until the next development came in 1961 from the laboratories of the Boehringer subsidiary Dr Karl Thomae GmbH, where Otto Thomae and Karl Zelle introduced orciprenaline (metaproterenol), which incorporated the ring system previously used by Eidebenz and Depner. Although this produced only a slight change in molecular topography, it conferred resistance to enzymic destruction since the catechol system had been replaced by one which, while similar enough to act on the catecholamine receptors in the bronchi, was now unable to fit on to the active site of the enzymes that deactivated isoprenaline and all other catecholamines, viz. sulfokinase in the gut wall and catechol O-methyltransferase elsewhere. Furthermore, as the hydroxyl substituents still remained on the ring, the drug was now too polar to enter the brain, hence patients did not experience the problem of insomnia found with ephedrine. Orciprenaline was marketed by Boehringer, as was fenoterol, an analogue prepared the following year. The selective action of fenoterol on the beta2-adrenoceptors of the lung was not recognised until after the revelation of similar selectivity in salbutamol. Its degree of selectivity was not as great as with salbutamol or terbutaline.
Reproterol was designed to combine the actions of orciprenaline and theophylline on the lung. Again, a selective action on the beta₂-adrenoceptors in the lung was not recognised until after the discovery of such selectivity in salbutamol. Reproterol turned out to have similar pharmacological properties to salbutamol and terbutaline.

Terbutaline

Terbutaline is an analogue of orciprenaline developed in 1966 by Kjell Wetterlin and Leif Svensson of the Draco division of Astra in Lund, Sweden. They replaced the isopropyl group in orciprenaline with a tertiary butyl group. This both enhanced potency and introduced a high degree of selectivity for the beta₂-adrenoceptors in the lung, which was comparable with that of salbutamol (see below). This meant that the heart rate was no longer increased as it had been with earlier bronchodilators. It has been the principal commercial rival to salbutamol, with little to distinguish between the two drugs in the clinic.

Bambuterol was developed as a long-acting prodrug of terbutaline. Plasma cholinesterase and oxidative enzymes promote the slow hydrolysis of the pharmacologically inert bambuterol into terbutaline, making it suitable for once-daily, oral dosage in the prophylaxis of asthma attacks.

Salbutamol (Albuterol)

An alternative approach to changing the position of one of the catechol hydroxy groups (as in orciprenaline) was taken in 1964 when Larsen and Lish of the Mead Johnson Research Center in Evansville, Indiana, replaced the hydroxyl group in the 3-position of the benzene ring by the chemically similar methanesulfonamide group to form soterenol. This was an effective bronchodilator.

David Hartley, David Jack, Lawrence Luntz and Alexander Ritchie of Allen & Hanbury’s in Ware (a division of Glaxo) developed salbutamol in 1967. The hydroxyl group of isoprenaline was deliberately replaced with a hydroxymethyl group to obtain resistance to destruction by catechol O-methyltransferase. As anticipated, the duration of action was lengthened by this manoeuvre, while potency remained high – unlike with orciprenaline.
However, a second modification consisted of the introduction of a tertiary butyl group into the molecule in place of the isopropyl group attached to the side chain nitrogen atom. This unexpectedly increased affinity for the beta2-adrenoceptors in the lung and reduced that for beta1-adrenoceptors in the heart. So marked was this selectivity in salbutamol that within only a few years it had displaced isoprenaline as the drug of choice for the control of asthmatic attacks.

Glaxo researchers later sought a longer-acting analogue of salbutamol that could confer protection against obstruction of the airways throughout the night so that the sleep of patients was not disturbed. They achieved this by attaching a non-polar chain on to the amine nitrogen, which caused the drug to partition mainly into the lipid bilayer of the bronchial cell membrane, with only a small amount remaining in the aqueous biophase. The lipid bilayer acted as a depot from which the drug was able to slowly diffuse out to produce continuing stimulation of the beta2-adrenoceptor. Salmeterol turned out to be not only longer acting but also 5–10 times as potent as salbutamol, permitting twice-daily inhalation to prevent bronchospasm.21 It was introduced clinically in 1988.

ADRENALINE ANTAGONISTS

The first drug to antagonise the effects of adrenaline on the heart was serendipitously discovered by Irwin Slater of Eli Lilly in Indianapolis while testing analogues of isoprenaline as potential long-acting bronchodilators. These were screened for their ability to relax tracheal strips that had been contracted by exposure to pilocarpine in order to simulate the bronchoconstriction of asthma. Between test runs, the strips were treated with adrenaline to ensure they were still responsive. However, those that had been exposed to dichloroisoprenaline did not relax when the adrenaline was added. This antagonism of adrenaline by dichloroisoprenaline was reported by Slater at a scientific meeting in 1957.22 Neil Moran, at Emory University in Atlanta, then requested samples of the new drug to investigate its effects on the heart. He found that while dichloroisoprenaline antagonised the changes in heart rate and muscle tension produced by adrenaline, it still mimicked the activity of the hormone to some extent.23

Moran’s report on the cardiac effects of dichloroisoprenaline interested James Black, who had been invited to join ICI Pharmaceuticals Division (now incorporated in AstraZeneca) at Alderley Park in Cheshire after seeking a grant from the company to further his investigations into coronary artery disease. Black believed that an alternative way of treating angina would be to find a drug that reduced the need of the heart for oxygen rather than merely increasing its supply through the use of vasodilators such as the nitrates. He realised that the heart rate was a major factor influencing the demand of the heart for oxygen, so had been seeking a drug to protect the hearts of patients with coronary disease against the effects of adrenaline and noradrenaline. On reading Moran’s paper, he realised it should be possible to find an analogue of dichloroisoprenaline devoid of intrinsic action when bound to the receptors in the heart (which belonged to the type described by Raymond Ahlquist as beta-adrenoceptors).24

John Stephenson synthesised the first effective beta-adrenoceptor blocker for Black in February 1960, by replacing the bulky chlorine atoms of dichloroisoprenaline with a second benzene ring to form pronethalol.25 The drug was active when given by mouth. A clinical trial on 30 patients confirmed pronethalol to be effective in angina, although mild side effects were noted. It was also somewhat short-acting. The therapeutic value, however, was not limited to the treatment of angina. Black’s belief, that it might prevent atrial fibrillation and atrial or ventricular tachycardias through diminution of the response to emotional or exercise-induced sympathomimetic activity, was confirmed in the clinic.26 What had not been anticipated, however, was the discovery that the drug had a marked hypotensive effect in anginal patients who had been taking it for several months. A subsequent report confirmed the value of pronethalol in reducing blood pressure in hypertensive patients.27
Shortly after, long-term toxicity testing in mice revealed that pronethalol could cause cancer of the thymus gland. Consequently, when the drug was marketed late in 1963, its use was limited to patients whose lives were seriously at risk. As a result, pronethalol was quickly superseded by another ICI product, propranolol. This, as well as all the other beta-blockers developed since then, retained the anti-anginal, anti-arrhythmic and antihypertensive properties of pronethalol.

Propranolol was synthesised in order to determine the effect of increasing the distance between the alcoholic hydroxyl group on the side chain and the aromatic ring of pronethalol. When it was found to be non-carcinogenic as well as ten times as potent as pronethalol it satisfied all the desired clinical criteria for a beta-adrenoceptor antagonist and so, in 1964, became the first one on the market licensed for general use. It remains in use as an anti-anginal, anti-arrhythmic and antihypertensive drug.

At AB Hässle in Göteborg, Sweden, analogues of dichloisoprenaline were investigated as potential anti-arrhythmic agents. Alprenolol emerged as an effective agent that could also be used in the management of hypertension. Oxprenolol, a successful beta-blocker developed by Ciba, retained some partial agonist activity when it interacted with beta₁ and beta₂-adrenoceptors. The stimulation of the beta₁-adrenoceptors in the heart meant that it tended to cause less slowing of the heart rate than those beta-blockers that did not exhibit partial agonist activity (i.e. intrinsic sympathomimetic activity). This feature was useful in some patients with mild peripheral vascular problems. Because of its stimulating action on beta₁-adrenoceptors in peripheral blood vessels, oxprenolol was less likely to induce a feeling of coldness in the extremities, an undesirable feature of some beta-blockers. However, the stimulation of beta₂-adrenoceptors in the lungs did not render oxprenolol sufficiently safe for use in asthmatic patients.

In timolol, not only was the aromatic system changed, but the N-isopropyl substituent was replaced by a tert-butyl group (cf. salbutamol). Timolol has been used as a beta-blocker both in the management of hypertension and by direct application to the eye for the reduction of intraocular pressure in chronic simple glaucoma. Other similar drugs are used in glaucoma.

A series of oxygenated compounds prepared from the dihydro analogue of propranolol were evaluated at the Squibb Institute for Medical Research in Princeton, resulting in the
introduction of nadolol. The low lipophilicity of nadolol prevented it from entering the central nervous system, hence patients did not experience the sleep disturbance and nightmares associated with more lipophilic beta-blockers. Another advantage of the low lipophilicity was reduced entry into liver cells, ensuring a longer duration of action than most beta-blockers.

Cardioselective Beta-blockers

Sotalol was synthesised as a potential beta-adrenoceptor agonist by Larsen of Mead Johnson in 1960. As Larsen had previously worked with sulfonamides, he synthesised sotalol in the belief that it would be worth replacing an acidic phenolic group of isoprenaline with an acidic sulfonamide group. Pharmacological evaluation indicated that sotalol not only relaxed tracheal, uterine and intestinal muscle but also blocked the tachycardia produced by the action of adrenaline on the heart in a similar manner to dichloroisoprenaline. Due to its polar nature, sotalol did not enter the brain to cause vivid dreams, nor undergo hepatic metabolism.

On learning of the enhanced safety margin associated with the use of sotalol, ICI chemists prepared a series of its analogues, which included practolol. Unexpectedly, it did not antagonise the peripheral vasodilation caused by isoprenaline in anaesthetised dogs, despite having the usual effects on the heart. It was the first time a beta-blocker was found that was relatively selective for heart receptors. This appeared to avoid a major clinical problem with beta-blockers, namely the risk of inducing bronchospasm in patients with asthma or obstructive airways disease through blockade of beta2-adrenoceptors in the lungs. Consequently, practolol was marketed in 1970 for use in asthmatic patients. Tragically, it caused a serious oculomucocutaneous reaction in a small number of patients on long-term oral therapy, some of whom were blinded. In 1975 its use was restricted to specialised hospital units. Fortunately, alternative drugs soon appeared and practolol was abandoned. Like practolol, these are relatively selective with regard to their action on beta2-adrenoceptors in the lung and on beta1-adrenoceptors in the heart.

Atenolol was found to be a relatively cardioselective analogue of practolol. For a long time it was the most frequently prescribed beta-blocker and the third-best-selling drug in the world (after ranitidine and cimetidine). Part of its success can be attributed to its low lipophilicity, which prevents it from entering the central nervous system.
Several alternatives to atenolol have been developed. May & Baker’s acebutolol did not have as much selectivity for cardiac receptors such as found with more recently introduced analogues of practolol. However, it retained partial agonist activity. Chemie Linz introduced celiprolol as the first relatively cardioselective beta-blocker in which the partial agonist activity was limited to the beta1-adrenoceptors. Labeltol, introduced by Allen & Hanbury, blocked the action of sympathomimetic amines both at beta-adrenoceptors and also at alpha1-adrenoceptors. Drugs exerting either of these actions are generally antihypertensive agents; hence it was believed that a compound such as labeltol would exert its effects on blood pressure at a lower level of adrenoceptor blockade.

The tendency of propanolol and other lipophilic beta-blockers to produce vivid dreams or even hallucinations in patients receiving large doses interested ICI chemists. To explore the nature of this phenomenon, they prepared analogues in which lipophilicity was further increased by linking the hydroxyl group to the nitrogen atom through a two-carbon chain to form a morpholine ring. The resulting compounds were psychoactive, and in 1969 viloxazine was patented as an antidepressant drug. It turned out to be a relatively non-sedating antidepressant that inhibited both noradrenaline and 5-HT reuptake in the brain. It was particularly of value in patients troubled by the anticholinergic and cardiac side effects of antidepressant drugs such as imipramine and amitriptyline.

OESTROGEN ANALOGUES

A variety of lipophilic ester prodrugs of oestradiol have been developed for formulation in oil to provide a long-lasting effect when administered as a depot injection. The first was oestradiol benzoate, which was patented by Schering-Kahlbaum of Berlin in 1936. The depot effect lasted for up to five days. After the ester had been slowly released from the site of injection, it entered the circulation and underwent hydrolysis in the plasma to release oestradiol. Other similar esters of oestradiol include the dipropionate, valerate, enanthate and undecanoate. However, the administration of oestradiol by long-acting injections of its esters in oil is now considered to be an inappropriate way of managing menstrual disorders or for hormone replacement therapy in the menopausal patient, since the slow decline in plasma oestrogen levels is unlike that of the natural menstrual cycle.

Oestradiol

Schering researchers Hans Inhoffen and Walter Hohlweg found that an ethinyl group introduced into the 17-position of progesterone conferred oral activity, so they decided to do likewise with oestrone to form ethinylestradiol. When injected into rats this showed similar potency to oestradiol, but given by mouth it turned out to be at least 20 times as potent as oral doses of the natural oestrogens. This was due to the acetylenic function at the 17-position of the steroid nucleus sterically blocking deactivation by liver enzymes. Ethinylestradiol remains in use for the treatment of menopausal symptoms, despite more than half a century having passed since its introduction into the clinic. It is also found as the oestrogenic component of oral contraceptives.
Synthetic Oestrogens

In 1932, Otto Rosenheim and Harold King had proposed that oestrogens had a similar structure to ergosterol, the structure of which had been established. Charles Dodds and Colin Leslie Hewett at the Middlesex Hospital in London joined forces with James Cook, an organic chemist working at the Cancer Hospital in London, to synthesise a tricyclic phenanthrene compound which they assumed would be an analogue of oestrone. This was found to have some oestrogenic activity. Although this tricyclic compound was the first substance of known constitution to exhibit oestrogenic activity, clinical application was out of the question. Nevertheless, its synthesis represented an important landmark.

Cook and Dodds next found oestrogenic activity in several other phenanthrenes. Then they discovered that the phenanthrene ring was not essential for activity. From this point onwards, they were able to demonstrate activity in a variety of compounds containing two benzene rings linked together via a short carbon chain, including diphenylethane and diphenylstilbene. One of the most potent was dihydroxystilbene. To establish whether both of its benzene rings were required for oestrogenic action, one was replaced by a methyl group. No complicated synthetic procedure was necessary to obtain the desired analogue as it was commercially available under the name ‘anol’, being cheaply prepared from the essential oil anethole. At first anol seemed to be as potent as any of the naturally occurring hormones. A letter was sent to the editor of *Nature*, in April 1937, to inform the scientific community of this remarkable development.

Within weeks, several workers had written to Dodds confirming his findings, while others wrote to say they had been unable to demonstrate any activity at all, even with high doses of anol. Walter Schoeller of the Schering–Kahlbaum laboratories then sent details of experiments that showed that some batches of anol were contaminated with an impurity formed during its formation from anethole. On crystallisation of crude anol from chloroform, this impurity remained in the mother liquor, proving to be highly oestrogenic. Dodds now collaborated with a group at the Dyson Perrins Laboratory in Oxford University, led by Robert Robinson, in an effort to synthesise this as-yet-unidentified impurity. They decided it could be a stilbene derivative formed by the condensation of two molecules of anol. Several such compounds were then prepared. Early in January 1938, the British researchers reported in *Nature* that one of these, subsequently known as ‘stilboestrol’ (diethylstilbestrol), was two or three times as potent as oestrone. Later, it was discovered to be almost as potent as oestriadiol when
injected, but around five times as potent when given by mouth. This was due to its enhanced resistance towards metabolic deactivation. As it was cheap to synthesise and well tolerated in patients, stilboestrol provided gynaecologists, for the first time, with a substance that was in plentiful supply and could be used to deal with oestrogen deficiency, especially in the menopausal patient. It was widely prescribed.

In his first publication about stilboestrol, Dodds drew attention to a structural resemblance between it and the natural oestrogens. Three years later, using X-ray crystallography, Dorothy Crowfoot established that the molecular dimensions were almost identical to those of oestradiol, especially with regard to the distance between the hydroxyl groups at either end of both molecules.

In 1971, two reports were published describing an increased incidence of vaginal and cervical adenocarcinomas in young women whose mothers had previously been given stilboestrol or similar synthetic oestrogens during the first trimester of their pregnancies in order to prevent unwanted abortion. Fear of a horrendous human tragedy that would have eclipsed even the thalidomide disaster led the US government to investigate the situation. It was found that the incidence of the adenocarcinomas was between 0.01 and 0.1% of those exposed. Up to five million American women are estimated to have taken the drug before its withdrawal, so up to as many as 5000 of their daughters could have developed adenocarcinomas. The situation is being monitored.

Paradoxically, stilboestrol is also used in the treatment of cancer. Following his isolation of testosterone, Ernst Laqueur and others administered it in unsuccessful attempts to treat enlarged prostate glands in elderly men. The belief that overgrowth of this gland must be due to diminished production of male hormone was challenged by Wugmeister, a Polish endocrinologist working in Milan. He had read of the detection of female hormones in male urine and also of the ability of such hormones to inhibit release of gonad-stimulating hormone from the pituitary gland. He suggested that a deficiency of female hormone might permit excessive release of gonadotrophic hormone from the pituitary, causing prostatic enlargement. In 1937, he put his hypothesis to the test by treating 23 patients with large doses of oestrone. His patients showed marked improvement, with clear reduction in prostate size. Wugmeister published his findings in Paris Medical, where they were seen by Pierre Kahle and Emile Maltry of the School of Medicine at Louisiana State University. They then carried out a similar study using the newly introduced stilboestrol. Their results, published in 1940, confirmed Wugmeister's claims and introduced this new form of therapy for benign prostatic overgrowth into the United States. They then initiated a trial of stilboestrol in prostatic cancer that was not completed until 1942, by which time others had already published evidence to show this was highly effective.

While Kahle and Maltry were carrying out their studies, Charles Huggins at the University of Chicago made a far-reaching chance observation. He had been collecting prostatic fluid from dogs for several years as part of an enquiry into the biochemistry of seminal fluid. Some of his experiments involved castrating the dogs prior to measuring the effect of testosterone injections on prostatic fluid production. It was in the course of one such study that Huggins noticed that castration caused regression of tumours that had spontaneously arisen in elderly dogs. After confirming that castration consistently caused shrinkage of prostatic tumours in dogs, Huggins realised that this indicated these tumours were hormone-dependent. He then administered stilboestrol in order to produce chemical castration. Again, regression of the tumours was observed. A year later, Huggins published the results of a trial of stilboestrol in patients with prostatic cancer that had spread into their bones. These results were most encouraging, the patients exhibiting marked regression of tumours and alleviation of pain. This was the first demonstration of a synthetic drug having induced an unquestionable improvement in a malignant disease. For this and his subsequent studies, Huggins was belatedly awarded the Nobel Prize for Medicine and Physiology in 1966.
Huggins’ pioneering investigations were soon followed by studies of the effects of hormones on various types of tumours, notably that conducted by Alexander Haddow at the Chester Beatty Research Institute of the Royal Cancer Hospital in London. From there, in 1944, he reported that stilboestrol and some of its analogues had beneficial effects in patients with breast cancer.53 This type of therapy was ultimately shown to be effective in up to 40% of postmenopausal women.

**Tamoxifen**

Following Dodds’ disclosure of the oestrogenic activity of diphenylethane and diphenyl-stilbene, John Robson and Alexander Schonberg of the Department of Pharmacology at the University of Edinburgh found that triphenylethylene had oestrogenic activity, although only about one-tenth-thousandth that of oestrone. The interesting feature, however, was that when it was given by mouth it was just as active as by injection, and the effects of a single dose lasted about one week. This was reported in 1937, and the following year the Edinburgh researchers showed that replacement of the sole ethylenic hydrogen atom with a chlorine atom increased potency two-thousandfold. Robson and Schonberg prepared more analogues, and early in 1942 reported that DBE, a bromine-substituted triphenylethylene with ethoxy groups attached to two of the benzene rings, was a possible alternative to stilboestrol.54 However, John Davies and his associates at ICI, who had followed the earlier reports by the Edinburgh group, were now working along similar lines. The outcome was that a British patent was awarded to ICI for the chloro-substituted triphenylethylene with methoxy groups on all of the benzene rings, namely chlorotrianisene.55 It was longer-acting than the natural oestrogens because its high lipophilicity resulted in deposition in fat tissue, from which it was slowly released.

Although chlorotrianisene was a weak oestrogen, it antagonised the ability of oestradiol to induce pituitary enlargement in rats. It was established that it was an anti-oestrogen which blocked oestrogen receptors in the hypothalamus. This interfered with feedback of oestrogen levels via the hypothalamic–pituitary axis.56 Chlorotrianisene was only a partial antagonist, retaining some intrinsic oestrogenic activity.

Clomiphene, now known to be a mixture of two geometric isomers, had only weak oestrogenic activity.57 It was a more potent anti-oestrogen than chlorotrianisene and was
capable of stimulating gonadotrophin release by inhibiting the negative feedback of gonadal oestrogens on the hypothalamus. As it had been found to cause enlargement of the ovaries in women, it was used to induce ovulation in women whose infertility was due to ovulatory failure. Experience has shown that considerable caution must be exercised when using clomiphene, otherwise multiple births may ensue.

Tamoxifen was synthesised by Dora Richardson of ICI Pharmaceuticals Division in 1962. When its two geometric isomers were evaluated by Michael Harper and Arthur Walpole, they found that while the cis isomer had oestrogenic activity, the trans isomer had only weak oestrogenic properties. Further investigation revealed the latter to act as an anti-oestrogen. As an analogue of stilboestrol, tamoxifen was active when given by mouth. It was therefore evaluated in rats as a postcoital oral contraceptive. However, when tested in women, it induced ovulation instead.

The anti-oestrogenic activity of tamoxifen was exploited in the treatment of oestrogen-dependent breast cancer, convincingly demonstrated in 1971 by a clinical trial at the Christie Hospital and Holt Radium Institute in Manchester. Two years later, tamoxifen was licensed in the United Kingdom for the treatment of breast cancer. Since then it has become established as the drug of choice for treating breast cancer in women who respond to hormone therapy. A large amount of evidence from randomised trials with tamoxifen has confirmed that it reduces the risk of recurrence of the disease and improves overall survival in both pre- and postmenopausal women, irrespective of whether or not they have received cytotoxic chemotherapy. However, it can cause serious side effects and there is a risk of contracting endometrial cancer.

**Formestane**

Formestane is an analogue of androstenedione that competes with it at the active site of aromatase enzyme to inhibit the final stage of oestrogen biosynthesis.

Formestane was introduced in 1984, being given intramuscularly as a depot injection every two weeks in patients in whom tamoxifen therapy had failed to control breast cancer. The introduction of orally active aromatase inhibitors has led to a decline in its use.

**PROGESTERONE ANALOGUES**

Progesterone was inactive by mouth because of rapid metabolism in the liver, but it was injected intramuscularly in the hope of preventing miscarriages during the early months of pregnancy. It is now generally believed that low levels of progesterone are not normally a cause of miscarriage. Progesterone later became available in the form of implants, pessaries and suppositories but it was expensive to produce and relatively large amounts had to be administered.
A potent, orally active analogue of progesterone was obtained by Leopold Ruzicka and Klaus Hofmann at the Zurich Eidgenossische Technische Hochschule, and also by Willy Logemann and his colleagues at the Schering laboratories in Berlin. These groups independently added potassium acetylide to dehydroepiandrosterone, a cheaply available, cholesterol-derived steroid intermediate. Their objective had been to introduce at the 17-position of the steroid nucleus a two-carbon acetylenic side chain which could subsequently be converted to the progesterone side chain. In the event, the stereochemistry at the 17-position of the resulting ethinylandrostenediol turned out to be the opposite of that in progesterone. The Schering group converted ethinylandrostenediol into ethisterone. This bore enough resemblance to progesterone to become established as a most successful orally active progestogen since disubstitution at the 17-position blocked metabolism by liver enzymes. Unfortunately, an inherent chemical resemblance to testosterone was responsible for virilising side effects of ethisterone and its analogues, a contraindication to use in pregnant women bearing a female foetus.

Norsteroids as Oral Progestogens

The first norprogestogen, in which the methyl group normally found in the 19-position was absent, was prepared in very low yield (0.7%) from the naturally occurring heart stimulant strophanthidin by Maximilian Ehrenstein at the University of Pennsylvania in 1944. His small supply of crude 19-norprogesterone was tested on two rabbits by Willard Allen (who with Oskar Wintersteiner had isolated progesterone ten years earlier). It was as active as the natural hormone. Further progress was not possible until Arthur Birch developed a practical synthesis of norsteroids at the University of Oxford in 1950. Using this procedure, Carl Djerassi and Luis Miramontes synthesised norethisterone (norethindrone) at the Mexico City laboratories of Syntex SA in 1951. It was marketed as a progestogen for the treatment of amenorrhoea, dysfunctional uterine bleeding and endometriosis.
Frank Colton of G.D. Searle & Company in Chicago synthesised a series of norsteroids that included norethynodrel, for which a patent was granted in 1954. Both norethisterone and norethynodrel were developed for the treatment of gynaecological conditions and it was only after Gregory Pincus found norethynodrel to be the ideal progestogen for incorporation in an oral contraceptive formulation that a lucrative market was created for norsteroids. Neither norethisterone nor norethynodrel had been synthesised with the aim of developing an oral contraceptive.

At the Wyeth Pharmaceutical Company in Pennsylvania in 1960, Herchel Smith, a former student of Birch, synthesised an analogue of norethisterone in which an ethyl group replaced the 18-methyl group. This new steroid, norgestrel, was more potent than either norethisterone or norethynodrel, its activity residing in the isomer known as levonorgestrel.

**Oral Contraceptives**

Leo Loeb revealed in 1911 that one of the functions of the mammalian corpus luteum was to inhibit ovulation. A quarter of a century later, A.W. Makepeace, George Weinstein and Maurice Friedman at the University of Pennsylvania successfully prevented ovulation in rabbits in oestrus by injecting progesterone for five days before mating. They attributed this outcome to pituitary inhibition and cited 15 previous reports of similar use of corpus luteum extracts of widely varying quality. This proof of the capability of progesterone to prevent conception was not exploited at that time for two reasons. Firstly, public opinion would have been against such a development and, secondly, the need to inject the progesterone would have severely limited its value. Yet, the following year Schering chemists developed the first orally active progestogen, namely ethisterone.

The investigation of potent progestogens as oral contraceptives did not take place until 13 years later when Gregory Pincus (see below), the author of another article that appeared in the same issue of the *American Journal of Physiology* as the University of Pennsylvania paper, became involved after being approached not by a pharmaceutical company but by Margaret Sanger, founder of the Planned Parenthood Federation of America.

The Federation was established by Sanger in 1916 under the name of the American Birth Control League. She dedicated her life to campaigning for the right of women to have access to effective means of contraception. She faced hostility on many fronts and was even imprisoned for her activities at a time when contraception was illegal in the United States. Sanger met Pincus over dinner in New York early in 1951, shortly after 75-year-old philanthropist Katharine Dexter McCormick, heiress to the International Harvester Company fortune, had written to Sanger asking her views on where financial support was most needed and what the outlook was for birth control research. Pincus, an authority on mammalian fertilisation, had established a small private laboratory in Worcester, Massachusetts, known as the Worcester Foundation for Experimental Biology. When asked by Sanger what the prospects were of science developing a perfect contraceptive, his reply so encouraged Sanger that she and McCormick decided to visit his laboratory. The result of that visit was that the Worcester Foundation received an initial $40,000 funding for research into oral contraception from McCormick. Over the next decade, that sum rose to 2 million dollars. This investment resulted in the development of what some consider may be the single most significant contribution of science to society in the twentieth century.

The initial approach taken by Pincus and his assistant Min Chueh Chang was similar to that taken in 1937 by Makepeace, Weinstein and Friedman except that, instead of injecting female rabbits with progesterone, they gave very large oral doses (more than 5 mg) in order to compensate for its extensive metabolism in the liver. Again, when the rabbits were subsequently placed in mating cages, none of them ovulated and no pregnancies occurred.
Shortly after completing his first experiments, Pincus happened to meet John Rock, a gynaecologist from Harvard University. Rock had been trying to stimulate the growth of underdeveloped ovarian tubes or uteri in infertile women by administering female hormones to mimic the stimulation in the growth of these organs produced by hormonal changes at the onset of pregnancy. In order to achieve the response he required, his patients were sequentially taking large oral doses of stilboestrol followed by progesterone, for three months. This regimen led to a total suppression of menstruation during treatment. Even though many of the women later conceived, this amenorrhoea was emotionally distressing. Pincus suggested to Rock that an alternative approach would be to give progesterone solely for three weeks and then withdraw medication so as to induce menstruation. Treatment could restart on the fifth day of the subsequent cycle.

When Rock tried this method, it proved acceptable to most of his patients. Several became pregnant after the three month course was completed. However, one in five of the women in the trial experienced ‘breakthrough bleeding’. This was due to the inadequacy of orally administered progesterone. Further, the hormone failed to maintain total suppression of ovulation for more than around 85% of the time that it was being taken, despite the use of 300 mg daily doses. It was evident that a more potent oral progestogen would be required, so in September 1953 Pincus invited leading pharmaceutical manufacturers to send him supplies of orally active progestogens that they had investigated. By the end of the year nearly 200 compounds had been examined at Worcester in both rabbits and rats. Fifteen were confirmed as potent ovulation inhibitors suitable for Rock to test in the clinic. These compounds were supplied to 50 infertile women. All were prevented from ovulating with doses in the order of 10–50 mg, a marked improvement over progesterone. In six of the patients, sufficient stimulation of their underdeveloped ovarian tubes or uteri occurred, permitting conception after the course of treatment was completed, the so-called ‘Rock rebound’. By now it was clear to Rock and Pincus that they had developed not only a remedy for one common type of infertility but also an effective oral anti-ovulatory therapy that could potentially satisfy Margaret Sanger’s expectations.75

The first large-scale clinical trial of a progestogen as an oral contraceptive began in Puerto Rico in April 1956. Norethynodrel was selected as it was the most potent of the anti-ovulatory compounds that had been examined. It was also free of androgenic activity. There were few reports of breakthrough bleeding during the early phase of the trial, but later numerous reports were received of this occurring towards the end of the treatment cycles. Thorough investigation revealed that these reports coincided with the introduction of purer batches of norethynodrel from the Searle laboratories. It was then discovered that the original batches had been contaminated with around 1–2% of the 3-methyl ether of ethinyloestradiol, from which the norethynodrel had been synthesised. This was a potent oestrogen, and when small amounts of it were deliberately incorporated in tablets containing pure norethynodrel, the problem of breakthrough bleeding was overcome. Later, this oestrogen was given the approved name of ‘mestranol’.

After two years, the preliminary results of the Puerto Rico trial were analysed. None of the 221 married women of proven fertility who had taken the tablets became pregnant, but there were several pregnancies among the small number of women who had dropped out of the trial, confirming the return of normal fertility. Finally, after examination of the clinical records of 1600 women who had received the tablets, the US Food and Drug Administration gave G.D. Searle & Company permission in May 1960 to market their combined progestogen–oestrogen oral contraceptive containing 9.58 mg of norethynodrel and 150 μg of mestranol. The following year, Schering introduced a similar product in Sweden containing 4 mg of norethisterone and 50 μg of ethinyloestradiol. The dose chosen for both these products was much higher than necessary. Today, for example, there is a combined oral contraceptive on the market containing 1 mg of norethisterone and 20 μg of
ethinyloestradiol. The diminution of the dose over the last 40 years has generally resulted in a reduction of side effects.

There was considerable concern during the early 1970s that chronic administration of oral contraceptives might cause some deaths as a result of cardiovascular changes. One theory was that residual androgenic activity in synthetic progestogens could be responsible for changes in the blood lipids (fats), leading to arterial disease, the chemical structures of progestogens having structural features in common with testosterone. In attempting to avoid this possibility, Organon introduced desogestrel, a compound synthesised in 1975 following reports that introduction of certain substituents in the 11-position of progestogens markedly enhanced potency.76 It was the first new progestogen to be launched in the United Kingdom for over a decade when it was marketed in combination with ethinyloestradiol in 1982. Within a year of its introduction, however, concern was also being expressed about the possible hazard of an increased risk of breast cancer in women who had been taking highly potent progestogens.

Mifepristone

At the laboratories of Roussel–UCLAF in Romainville, France, Georges Teutsch developed a synthetic route for 11β-substituted steroids in 1975. He used this to synthesise a series of novel compounds that were examined as potential glucocorticoids. The two compounds that were found to bind most tightly to the rat hepatic glucocorticoid receptor during in vitro studies, RU 25055 and RU 25593, were unexpectedly found to be wholly devoid of activity in another experimental screening model in which glucocorticoid activity was correlated with induced tyrosine amino transferase in rat hepatoma tissue cells. Further investigation revealed that both test compounds antagonised the action of dexamethasone in this model. The possibility that this could be due to antiglucocorticoid activity was considered, but other possible explanations for the behaviour of the compounds also existed. A variety of tests for antiglucocorticoid activity proved inconclusive.

In 1980, specific screens for antiglucocorticoid activity were established by the company following a renewal of interest in the activity of the two compounds synthesised by Teutsch. Several more were synthesised, the sixth of which totally blocked the activity of dexamethasone while still being devoid of glucocorticoid activity. When this compound, RU 38486, was subjected to evaluation in a battery of pharmacological models over the next few months, it consistently behaved as an antiglucocorticoid.

Teutsch knew that in the initial receptor binding studies of his 11β-substituted steroids, the two antiglucocorticoids had also tightly bound to the progesterone receptor. He therefore requested that they be screened to determine their effect at this receptor. When absolutely no activity on the rabbit endometrium was demonstrated, the possible parallel with the results in the glucocorticoid models immediately struck him. At the beginning of 1981, his suspicion that RU 38486 was an antiprogestin was confirmed.77 Further evaluation in rats revealed this to be a potent antagonist of progesterone since it deformed the receptor and thus prevented the activation that normally ensued after progesterone was attached. RU 38486 was given the name ‘mifepristone’ and was patented in 1982.78
The potential of an antagonist of a hormone that was essential for the maintenance of pregnancy was obvious. Tests on small animals and primates confirmed that administration of mifepristone daily for four days interrupted early gestation. In a trial at the University Hospital in Geneva in 1982, 11 volunteers who were between six and eight weeks pregnant were given 200 mg of mifepristone by mouth daily for four days. Abortion was complete in 3–5 days in eight cases and in eight days in another. In two cases, the procedure failed. These two failures were addressed by taking into account one of the important effects of progesterone, which is to render the uterus less responsive to excitatory agents such as prostaglandins. Since mifepristone made the uterus more susceptible to the action of prostaglandins by antagonising progesterone, the supplementation of it with a prostaglandin analogue to stimulate the uterus to contract was investigated. Several studies found that when a single dose of 600 mg of mifepristone was followed two days later by such an analogue, misoprostol, more than 95% of early pregnancies aborted. This combination of drugs was licensed for use in France in 1987 and in the United Kingdom four years later. Its introduction, not unexpectedly, was surrounded by controversy and it was not licensed for use in the United States until 2000.

Mifepristone proved to be of no clinical value as an antiglucocorticoid since on continued administration its effects were overcome by an increase in corticotrophin and hydrocortisone levels arising from biofeedback via the hypothalamic–pituitary axis.

Medroxyprogesterone Acetate

In 1954, Karl Junkmann of Schering AG reported that the acetylation of the 17-hydroxyl group of ethisterone provided a derivative suitable for formulating in oil for injection intramuscularly as a depot medication. There resulted widespread interest in preparing the acetates (and other esters) of various hydroxy-steroids. One such ester, Upjohn’s 17-acetoxyprogesterone, proved to be a promising progestogen even though its hydroxy precursor was inactive. Unfortunately, it turned out that no significant prolongation of action was obtained by formulating it in oil. The Upjohn researchers, however, made the unexpected discovery that their acetoxo derivative was orally active, an observation that had been missed by the Schering group, who were primarily interested in the oil solubility of such esters. Several manufacturers then competed to prepare the 6-methyl derivative of 17-acetoxyprogesterone. Priority of discovery for this derivative, subsequently known as medroxyprogesterone acetate, went to Upjohn in the United States for a patent application submitted on 23 November 1956. Syntex, however, submitted its application in France on 8 September 1956.

Medroxyprogesterone acetate proved to be around 25 times as potent as ethisterone. When formulated as an oily injection, it formed a deposit of steroid in the body near the site of intramuscular injection. Small quantities of drug were then slowly released over a period of several months to give prolonged contraceptive cover.
Vladimir Petrow of the British Drug Houses oxidised medroxyprogesterone acetate, a drug he had developed as an oral contraceptive, to introduce an extra double bond at the 6-position. He did not expect this to have useful progestational activity, since the analogous derivative of progesterone was less potent than progesterone itself. Surprisingly, routine screening in rabbits revealed this new compound, megestrol acetate, to be the most potent ovulation inhibitor discovered up to that time (1958).82 Syntex and E. Merck AG both found an alternative way of blocking metabolism at the 6-position, namely by inserting a chlorine atom. They independently prepared chlormadinone acetate.83,84

TESTOSTERONE ANALOGUES

Following the introduction of oestradiol benzoate in an oily depot formulation, testosterone phenylacetate was likewise prepared for intramuscular injection.85 It has been replaced by a variety of other testosterone esters used for the same purpose, either on their own or as mixtures, including testosterone propionate, which is a shorter-acting ester formulated as an oily injection for administration two or three times a week. It is also formulated as a component of mixtures containing several esters with varying duration of action, such as the phenylpropionate, enanthate and undecanoate.

Testosterone undecanoate is also formulated in capsules given by mouth. Because of its short half-life, thrice-daily dosing is required.

Anabolic Steroids

At the University of Rochester in 1931, John Murlin tried to determine whether the higher metabolic rate in the male than the female was related to hormonal activity. The administration of androgenic male urine extract to castrated dogs appeared to increase the basal metabolic rate, but this could not be confirmed by Murlin. Charles Kochakian and Murlin then refined the study by examining the effect of a potent androgenic male urine extract on the abnormal levels of nitrogen (derived from protein metabolism) to be found in the urine of castrated dogs.86 This extract had no significant effect on the metabolic rate, but it did reduce urinary nitrogen levels, indicating that protein metabolism had been decreased by
the androgen. This was followed by an increase in the body weight of the dogs. When testosterone became available, Kochakian found that it had a similar influence on nitrogen balance. A similar effect with testosterone in patients with endocrine deficiencies was subsequently reported by Kenyon. Due to its hormonal effects, however, testosterone could only be administered as a muscle-building drug to mature male patients.

In 1948, G.D. Searle & Company initiated a long-term project to exploit the muscle-building properties of testosterone, hoping to obtain an anabolic agent free of hormonal activity. The screening procedure compared any increase in the weight of the seminal vesicles and the prostate glands of castrated rats with that of the levator ani muscle. In an ideal anabolic agent, only the weight of the latter would increase. This screen was applied to more than 1000 steroids over a 7 year period. Eventually, when Colton's norsteroids were examined in 1955, norethandrolone emerged as having similar anabolic activity to testosterone, with only one-sixteenth the potency as an androgen. Furthermore, it was orally active since the alkyl substituent at the 17β-position stabilised the 17β-hydroxyl substituent against oxidation in the liver. After a satisfactory clinical trial, norethandrolone was released for use in debilitated patients in the expectation that enhanced utilisation of protein would build up wasting muscles. This expectation has been realised only to a very limited extent, even with newer drugs. Growing concern about the risk of cholestatic jaundice resulted in the withdrawal of norethandrolone from the market in the 1980s.

Nandrolone was one of the norsteroids originally synthesised by Birch. As it was an analogue of norethandrolone, it had anabolic activity. Unlike norethandrolone, it does not possess a 17α-ethyl substituent to stabilise the 17β-hydroxyl substituent against oxidation in the liver. Consequently, nandrolone is inactive by mouth. It is administered as an injection in oil. Nandrolone decanoate is used in an oily depot injection.

Finasteride

An inherited condition in which apparently hermaphrodite children develop into males at puberty was found, in 1974, to be caused by a deficiency of 5α-reductase, the enzyme that converts testosterone to the more potent dihydrotestosterone. Subsequent studies revealed that while most organs were responsive to testosterone and thus developed normally as testosterone levels rose at puberty, some were responsive only to dihydrotestosterone. The lack of development of the prostate gland throughout life in those with the 5α-reductase deficiency syndrome was attributed to the lack of responsiveness of the prostate to testosterone.
Chemists at the Merck Research Laboratories postulated that 5α-reductase inhibitors could be of value in the treatment of benign prostatic hyperplasia, an overgrowth of prostatic tissue commonly found in males over the age of 50. The resulting enlargement of the gland causes obstruction of urinary flow through the urethra, frequently requiring surgical intervention. In 1985, they found finasteride, an azasteroid (i.e. incorporating a nitrogen atom), to be a potent, orally active inhibitor of 5α-reductase.92 It has proved to be of value in the treatment of benign prostatic enlargement, where it can stop progression of the disease in mild to moderate cases, even to the extent of reducing the volume of the prostate. It thereby avoids the need for surgery so long as treatment is continued.

CORTISONE ANALOGUES

When cortisone and hydrocortisone were introduced, there was no demand for any new analogues to be prepared. Both hormones seemed to offer all that could be expected of corticosteroid therapy, bearing in mind that the activity of a number of existing corticosteroids had already been carefully evaluated in several laboratories. Sarett and colleagues pointed out that the decade preceding the introduction of cortisone witnessed the introduction of a wide range of vitamins. Their success had strengthened the long-held view that natural products were inherently superior to synthetic drugs, thereby discouraging the quest for synthetic analogues of cortisone.93 A complementary viewpoint to that of Sarett can, with hindsight, be taken today: the successful development of therapeutically significant synthetic corticosteroids was a key stimulus for medicinal chemists to synthesise analogues of other natural products. Nevertheless, the first two analogues of cortisone that were introduced were, in fact, the unexpected products of attempts to find novel means of synthesising the natural hormone.

The first useful analogue of hydrocortisone was discovered when the Squibb chemist Joseph Fried attempted to synthesise hydrocortisone from its isomer epicortisol. These two compounds differed only in the stereochemical orientation of the hydroxyl group at the 11-position of the molecule. In order to achieve the conversion, Fried first had to introduce a bromine atom at the 9-position. Routine screening revealed that 9β-bromoepicortisol had one-third of the activity of cortisone. As epicortisol was feebly active, it appeared that bromination had increased potency. Fried next prepared 9α-halogenated analogues of hydrocortisone, the potency of which turned out to be inversely proportional to the size of the halogen substituent. This indicated that fluorine would be an ideal halogen to use in preference to the bulky bromine. When 9α-fluorohydrocortisone was tested, it was found to be about 10 times as potent as cortisone in relieving rheumatoid arthritis.94 Unfortunately, the salt- and water-retaining (mineralocorticoid) activity was increased by a factor of 300–800. This showed – for the first time – that molecular manipulation might alter the balance of mineralocorticoid and glucocorticoid activity in corticosteroids. It also meant that patients became moon-faced and oedematous, hence its clinical role was limited to the management of adrenal insufficiency in Addison’s disease or hypopituitarism. The new steroid received the approved name of fludrocortisone.

During an attempted synthesis of hydrocortisone, chemists of the Schering Corporation in Broomfield, New Jersey, protected the sensitive 11-hydroxyl group of the steroid ring by
acetylation. At the penultimate stage in the synthesis, what was expected to be routine removal of the 11-acetate by chemical hydrolysis instead resulted in decomposition of the molecule. The only way round this was to attempt bacterial hydrolysis. Unexpectedly, in addition to ester hydrolysis, oxidation of the steroid ring A also resulted in the introduction of a second double bond. Two novel steroids were isolated, namely prednisone and prednisolone. A preliminary clinical trial of them at the National Institutes of Health indicated that both compounds were about five times as potent as hydrocortisone in the treatment of arthritis and inflammatory conditions, yet no more likely to cause salt retention. The duration of action of both was about three times as long as that of hydrocortisone. Encouraged by this outcome, the company took the decision to risk pouring $100,000 worth of cortisone into a large fermentation tank. The gamble paid off handsomely when prednisone generated more than $20 million in sales for Squibb within a year of its introduction in 1955. For many years, prednisone was as widely prescribed as prednisolone, but ultimately the latter compound was preferred once it was generally realised that it was also the active metabolite of prednisone.

Because of its diminished effect on mineral balance, prednisolone has remained the most commonly prescribed steroid for oral anti-inflammatory therapy. Its instant success in the market-place undermined much of the effort of several leading pharmaceutical companies to develop their own syntheses of cortisone or hydrocortisone. However, now that it was known that the potency can be increased by the introduction of a 9α-fluoro substituent and also that glucocorticoid activity can be enhanced through the placing of an extra double bond in ring A, the pharmaceutical industry rapidly set about preparing more corticosteroid analogues. One of the first, methylprednisolone, was designed in the wake of a report that hydrocortisone was metabolically deactivated by oxidation in the liver to its 6-hydroxy derivative. This gave researchers at Upjohn the idea of blocking the metabolism of prednisolone by placing a methyl substituent on the vulnerable 6-position to hinder the fit of the steroid at the surface of the oxidative enzymes. Methylprednisolone proved to be slightly more potent than prednisolone.

Following their isolation from the urine of a boy with an adrenal tumour of corticosteroids substituted with a hydroxyl group at the 16α-position, researchers at Lederle Laboratories, led by Seymour Bernstein, synthesised several steroids of this type so that their biological activity could be examined. They found that the 16α-hydroxyl group decreased mineralocorticoid activity, an observation that was to have far-reaching implications. It was immediately exploited to overcome the major shortcoming of fludrocortisone by also placing a second
double bond in ring A. This resulted in the introduction of triamcinolone at the beginning of 1958. Triamcinolone was also synthesised at the same time by Squibb, so a cross-licensing agreement was made between the two companies in order to avoid patent difficulties.

Clinicians found triamcinolone to be largely free from mineralocorticoid activity, while still as potent an anti-inflammatory agent as prednisolone. Yet it did not live up to its early promise. There was a distinct tendency to cause nausea, dizziness and other unwelcome effects.

In an attempt to hinder metabolic reduction of the carbonyl at C20 in the side chain attached to corticosteroids, Merck chemists employed a technique previously developed during an investigation when it had been found that introduction of a 16\(\alpha\)-methyl group hindered the rapid metabolic reduction of the carbonyl function at C20 on the side chain. They achieved their objective of lengthening the duration of action, but of even greater significance, however, was the discovery that the 16\(\alpha\)-methyl group not only acted similarly to a hydroxyl group in that position by depressing mineralocorticoid activity but it also enhanced anti-inflammatory activity. The outcome was the introduction of dexamethasone in 1958. It was six times as potent as prednisolone and 25 times as potent as hydrocortisone, with a duration of action of 36–54 hours (hydrocortisone being about 8–12 hours). Dexamethasone is used when long-term medication with a potent systemic corticosteroid is required, as well as in shock and cerebral oedema. Merck also introduced betamethasone, the isomer of dexamethasone in which there was a 16\(\beta\)-methyl group. This alteration made little difference to the biological activity, both drugs being six or seven times as potent as prednisolone, with practically no salt-retaining activity at all. Neither of these drugs had the unwelcome side effects previously seen with triamcinolone.

**Topical Corticosteroids**

The first reports of corticosteroids being applied to the skin appeared in 1952. Since then, this has become a popular means of treating inflammatory conditions such as psoriasis. Although hydrocortisone and its acetate were initially used, the range of products available for treating severe conditions was widened by the development of highly lipophilic, potent compounds. The first of these was triamcinolone acetonide, introduced by Lederle.
The fact that the acetonide was easily prepared by stirring a suspension of triamcinolone in acetone containing a trace of perchloric acid encouraged other companies to develop similar compounds for topical treatment of severe dermatological conditions. Syntex, for example, placed a fluorine atom at the 6-position of triamcinolone to prevent metabolic oxidation and thereby increase potency in fluocinolone acetonide.\textsuperscript{102} Squibb introduced halcinonide, a chlorine-containing analogue of triamcinolone acetonide.\textsuperscript{103} As this was a highly potent topical anti-inflammatory agent, it was reserved for the treatment of severe skin conditions that did not respond to milder agents. A British patent on fluocinonide, which was the acetate of fluocinolone acetonide, was granted to the Olin Mathieson Company in 1963, while Syntex obtained a US patent for the same compound the following year. Several other potent corticosteroids have been marketed.

**Inhaled Corticosteroids**

In 1958, Merck applied for a patent on beclomethasone, a glucocorticoid with similar potency to dexamethasone.\textsuperscript{104} Although this was granted four years later, the drug does not appear to have been marketed. However, in the early 1970s Glaxo introduced beclomethasone dipropionate in an aerosol for the treatment of asthma.

Beclomethasone dipropionate represented a major advance over oral administration of drugs such as prednisolone since the effects were largely confined to the lungs.\textsuperscript{105} Since then, other topical steroids have been similarly formulated, including budesonide\textsuperscript{106} and fluticasone propionate.\textsuperscript{107} With the newer agents, very little of the drug was absorbed, which was of advantage since up to 80\% of any inhaled dose of any drug may be swallowed.
ALDOSTERONE ANALOGUES

Spironolactone was prepared in 1959 by researchers at the laboratories of G.D. Searle & Company in Chicago as an antagonist of aldosterone, which blocks the aldosterone receptor. As it increased the excretion of sodium ions, chloride and water while retaining potassium and magnesium ions, spironolactone was used as a potassium-conserving diuretic, either alone or in combination with other diuretics. In recent years a growing awareness of the role of aldosterone in cardiovascular disease has led to the use of spironolactone in heart failure.

Canrenone is an active metabolite of spironolactone, which was synthesised at the same time as spironolactone. When parenteral spironolactone treatment is required, canrenone is used in the form of the potassium salt of the open-chain free acid formed from the lactone, viz. potassium canrenoate. This is rapidly converted \textit{in vivo} to canrenone. Canrenone is better tolerated than spironolactone itself, presumably because of the absence of the thiol ester function.

POSTERIOR PITUITARY HORMONE ANALOGUES

Sandoz researchers Stephan Guttmann and Roger Boissonnas prepared analogues of oxytocin and vasopressin in which one or more amino acids were replaced. This initially led to the introduction in 1960 of felypressin as a vasoconstrictor. It is mainly used in combination with prilocaine to prolong local anaesthetic action.

Removal of the free amino group in position 1 of vasopressin was shown to increase antidiuretic activity 2–3 times. Boissonnas then discovered that when d-arginine was also put in place of the l-arginine in position 8, pressor activity was reduced by a factor of more than 1500. In the resulting compound, desmopressin, the antidiuretic activity was almost 4000 times greater than the pressor activity and hence it had no vasoconstrictor action in clinical use. It was also longer-acting than vasopressin. When first marketed for the treatment of diabetes insipidus, desmopressin was administered either by injection or by nasal spray. Tablets are now available, but because of enzymatic destruction they have only one-hundredth of the bioavailability of the hormone administered parenterally. Nonetheless, the introduction of a tablet containing a peptide is significant since such molecules are usually completely digested.
Terlipressin was developed as a prodrug of porcine lysine–vasopressin for intravenous administration as an emergency measure to stop bleeding from oesophageal varices. These varicose veins in the lower part of the oesophagus occur as a consequence of portal hypertension in patients with cirrhosis of the liver. In this situation, it is the vasoconstrictor action of vasopressin that is exploited as the glycyl groups are enzymatically cleaved from terlipressin to give a sustained release of the active hormone over a period of 4–6 hours after injection.

HISTAMINE H₂ RECEPTOR ANTAGONISTS

The early antihistamines (see Chapter 28, section on Classical Antihistamines) were unable to antagonise the release of gastric acid caused by the action of histamine, leading to the suggestion by Bjorn Folkow and Georg Kahlson in Gothenburg that there might be more than one type of histamine receptor. This view was reinforced by experiments with alkyl-substituted histamine analogues that did not have equal activity on histamine receptors in different tissues. James Black thought there might be a parallel between the situation regarding histamine and that which pertained for adrenaline at the outset of his previous work on the beta-blockers. Having recently moved to the Smith Kline and French Research Institute in Welwyn, Hertfordshire, he speculated as to whether the failure of antihistamines to prevent gastric acid release might be due to their inability to block a different histamine receptor. This raised the possibility that it could be possible to treat gastric ulcers with a new type of antihistamine. The receptors that were blocked by the classical antihistamines were later given the name histamine H₁ receptors, while those in the stomach that were not affected by classical antihistamines were to be named histamine H₂ receptors.

In 1964, Black and Michael Parsons set up an assay procedure to detect the proposed new type of antihistamine, measuring the effect of experimental compounds in preventing the production of acid in the stomach of anaesthetised rats. Robin Ganellin was in charge of the project to find the desired antagonist, assisted by Graham Durant and John Emmett. For four years they synthesised and tested over 200 histamine analogues, but none met their objective of fitting the histamine receptor in the stomach without triggering acid release. The first breakthrough came with a compound that had been synthesised at the start of the project, namely guanylhistamine. Its activity as a blocker of histamine-induced acid release had been overlooked because it mimicked the action of histamine by producing some acid release. This was an example of ‘partial agonism’ as previously found with dichloroisoprenaline. Within a few days of this being recognised, Emmett found that isosteric replacement of a nitrogen by a sulfur atom to form the isothiourea analogue provided enhanced potency as a blocking agent, although the acid-releasing activity still remained.
Eventually it was realised that so long as the side chain could ionise to acquire an electronic charge, as histamine did, then the acid-releasing activity would remain. This was avoided by replacing the ionisable moiety with a thiourea function. The polarity of the thiourea group resembled that of the amino group in histamine, facilitating the fit of the drug to the gastric receptor. This compound, known as burimamide, was tested in 1970 and proved to be solely a histamine blocker which, on injection, prevented the release of gastric acid. Burimamide inhibited the release of gastric acid in animals and man, but it lacked sufficient potency to be administered by mouth.

By careful consideration of electronic effects influencing the disposition of the hydrogen atom attached to one or other of the nitrogens in the imidazole ring, a derivative was obtained in which an electron-donating methyl group induced protonation on the adjacent nitrogen atom. This was metiamide, which was ten times as potent as burimamide and so could be administered by mouth. Unfortunately, during early clinical trials involving 700 patients, a few cases of a blood disorder, granulocytopenia, were reported and the drug was abandoned even though the condition was reversible.

Suspicion fell on the thiourea group, so other polar groups were examined, resulting in the development of cimetidine, which was synthesised in 1972. The Smith Kline and French researchers found that either a nitrile or a nitrovinyl group attached to the guanidino nitrogen atom withdrew the electrons that would otherwise confer basicity and resulted in the molecule being ionised in the body. Cimetidine was shown to be effective in reducing acid release in animals and was introduced into clinical practice in 1976, 12 years after Black’s initial experiments. It was a remarkable achievement which even today still represents medicinal chemistry at its best. Ganellin has written a detailed account of the discovery of cimetidine. In the clinic, cimetidine fulfilled its early promise of healing peptic ulcers by reducing gastric acid release. It soon became the world’s best-selling drug with sales exceeding $1000 million by 1983. It was eventually overtaken by its analogue ranitidine.

Cimetidine occasionally caused problems through its ability to enter liver cells and bind via its nitrile function to the haem moiety in microsomal cytochrome P450. This interfered with the oxidative metabolism of a variety of drugs. For those drugs with a wide margin of safety it was not a problem, e.g. benzodiazepines and beta-blockers. However, when drugs with a narrow therapeutic window were being taken, then serious problems could arise, e.g. phenytoin, warfarin or theophylline. Also, some elderly patients experienced confusion after taking cimetidine due to its ability to enter the central nervous system. Some male patients developed gynaecomastia or the even rarer impotence from binding of cimetidine to androgen receptors, but this was dose-related and rarely occurred at normal dose levels.

During the early stages of the development of cimetidine, Black and his colleagues investigated a variety of alternatives to the imidazole ring but none proved effective. Smith, Kline and French had been remarkably non-secretive by publishing their ongoing work which was to lead to the development of cimetidine. This enabled a Glaxo research team led by Roy Brittain and David Jack to tackle the problem posed by the lack of oral activity of burimamide. They began by preparing tetrazole isosteres of the imidazole ring in burimamide since the company had considerable experience of tetrazole syntheses. This resulted in the preparation of AH 15475, a tetrazole that had similar potency to burimamide, but in addition exhibited evidence of oral activity in the dog.
Although many tetrazoles were synthesised and tested, no compound emerged as superior to AH 15475. By this time, early 1976, Smith Kline and French had published their first reports on metiamide and its successor cimetidine. The project was continued by Glaxo because it was recognised that the aminotetrazole ring of AH 15475 ($pK_a$ of 1, approximately) lacked the overt basicity of the imidazole ring in burimamide ($pK_a$ of 6.8), yet the compound was just as potent. This indicated that non-basic heterocycles might be considered as alternatives to imidazole.

The first heterocycle that was utilised was 2-furfuryl mercaptan since supplies were commercially available. Incorporation of the side chain of metiamide gave AH 18166. This was insoluble and thus gave erratic results during biological evaluation. The problem was overcome by taking advantage of the ability of furans to undergo the Mannich reaction, resulting in the formation of an analogue with a basic side chain that would confer water solubility by permitting salt formation, namely AH 18665. This proved to be as potent as metiamide in various in vitro tests. However, concern that AH 18665 would produce agranulocytosis, as had metiamide, led the Glaxo researchers to test its cyanoguanidine analogue, AH 18801. This proved to be very similar to cimetidine in its pharmacological profile. When this turned out to have a low melting point and lacked crystallinity, the Glaxo chemists recalled their previous experience with one of the tetrazole analogues in which replacement of the cyano substituent with a nitrovinyl group overcame this very problem. To their dismay, this did not produce the crystalline compound they had hoped for when a similar alteration was made to the structure of AH 18801. Equally unexpected was the discovery that this new nitrovinyl compound was much more potent than cimetidine. With the approved name of ranitidine, it was destined to become the best-selling drug in the world for many years to come. A detailed account of its discovery has been published.

Ranitidine did not bind to cytochrome P450 since it lacked the nitrile function as it had been replaced in the attempt to obtain a crystalline compound. Furthermore, insertion of the basic dimethylamino substituent to permit water solubility had made ranitidine more polar than cimetidine. This rendered it less likely to enter the brain and cause confusion. Nor did ranitidine produce breast enlargement or impotence from binding of cimetidine to androgen receptors. As many physicians preferred to prescribe it rather than cimetidine because of the reduced likelihood of side effects, for many years it was the best-selling drug in the world, with cimetidine taking second place.
In famotidine, the inherent basicity of the terminal guanidine function is removed by the incorporation of an electron-withdrawing sulfonamido group rather than a nitrile or nitrovinyl group. Famotidine has about 20 times the potency of cimetidine as an H₂ antagonist and is longer-acting, but for most patients this is of little significance since, at equipotent doses, H₂-blockers are all equally effective in the treatment of peptic ulcers. Nizatidine has nine times the potency of cimetidine as an H₂ receptor antagonist, but again this is of little clinical significance.

5-HT ANALOGUES

Once 5-hydroxytryptamine had been implicated in both inflammatory processes and as a chemotransmitter in the central nervous system, pharmaceutical companies showed interest in its potential for therapeutic exploitation. In more recent times, interest has centred on the development of agonists and antagonists displaying receptor selectivity.

Indometacin

Researchers at the Merck Institute for Therapeutic Research, West Point, Pennsylvania, found indometacin to be the most efficacious among 350 indole compounds screened in animals because of the possibility that serotonin might be involved in the inflammatory process. It proved to be both the most potent and the most toxic of the non-steroidal anti-inflammatory drugs. As side effects such as dizziness, headache or gastrointestinal disturbances were common, Merck subsequently introduced sulindac, a prodrug that was metabolised in the liver to form an active metabolite by conversion of the sulfoxide function to a sulfide. It lacked the high potency of indometacin but was well tolerated, apart from gastrointestinal disturbance occurring in some patients.

E. Merck and Company in Germany developed acemetacin, the glycolic acid ester of indometacin, in an attempt to provide a safer drug. However, as acemetacin was
metabolised to form indometacin, it retained its disadvantages, although gastrointestinal side effects may possibly have been reduced.

**Benzydamine**

![Structural formula of Benzydamine and Indoramin]

At the research laboratory of Angelini Francesco in Rome, 34 indazoles were synthesised as isosteric analogues of serotonin in which one of its ring nitrogen atoms had been moved.\(^{129}\) Some of these incorporated molecular features previously found in antihistamines. As little was known about the activity of indazoles, the compounds were screened for anticonvulsive, antispasmodic, analgesic, anti-inflammatory and anaesthetic activity. Several were active and had low toxicity, with benzydamine emerging as an analgesic, anti-inflammatory agent. It was incorporated in topical preparations such as creams and mouthwashes.

Indoramin was synthesised by John Archibald, John Cavalla and their colleagues at the John Wyeth British laboratories near Maidenhead, following the company's discovery that compounds featuring the indolylethylpiperidine moiety reduced blood pressure.\(^{130}\) It was marketed in the United Kingdom in 1981 and is an alpha D\(_1\)-adrenoceptor antagonist with potent antihypertensive activity.

![Structural formula of Zolpidem]

Zolpidem\(^{131}\) is an indole isostere developed by Jean-Pierre Kaplan and Pascal George of Synthélabo at Vitry-sur-Seine, France. It is a hypnotic that acts primarily at benzodiazepine omega-receptors. Its hypnotic properties are similar to those of the benzodiazepines, but with a less impressive safety profile.

**Selective 5-HT Agonists and Antagonists**

John Gaddum at the University of Oxford discovered that 5-HT act on the isolated guinea pig ileum by two distinct mechanisms.\(^{132}\) The first was directly on smooth muscle, while the
second was mediated through the release of acetylcholine from cholinergic nerve endings and was blocked by morphine. The first mechanism was described as involving the serotonin D-receptor, whereas the second was said to involve the serotonin M-receptor. The M-receptor was later renamed as the 5-HT₃ receptor. A variety of indole analogues that were 5-HT antagonists had been developed by the late 1970s, yet none of them had selectively blocked the 5-HT₃ receptor.

Glaxo researchers recognised that existing methods of testing, such as on the guinea pig ileum, were indirect insofar as they depended upon the detection of the release of one or more neurohormones. Considering this to be unreliable, they instead measured the depolarisation of the rat vagus nerve, a direct indicator of 5-HT₃ receptor stimulation. This enabled them to identify 5-HT₃ receptor blockers in amines derived from indolylpropanone. After an initially promising series of imidazoles proved to be inactive by mouth, the decision was taken to limit the flexibility of the indolylpropanone side chain by linking it to an adjacent position in the indole ring, thus forming a third ring to give a tetrahydrocarbazolone. When this gave encouraging results, the corresponding analogue of the most promising of the earlier imidazoles was synthesised. It proved active by mouth and in 1990 became the first 5-HT₃ antagonist to be introduced into the clinic, with the approved name of ‘ondansetron’. It was prescribed as an anti-emetic agent to control vomiting induced when chemotherapy releases 5-HT from enterochromaffin cells in the upper gastrointestinal tract. The released 5-HT then acts on both local 5-HT₃ receptors as well as on those in the chemoreceptor-trigger zone of the brain to stimulate the vagus nerve and so cause vomiting.

Investigations by John Fozard and his colleagues at the Merrell Dow Research Institute in Strasbourg in the late 1970s revealed that cocaine (and metoclopramide) blocked 5-HT₃ receptors. Sandoz researchers then tried to find potent and selective blockers based on the structure of 5-HT itself. They began by extending the ethylamine side chain and then made further analogues in which the side chain nitrogen was provided by incorporation of the tropine ring system (which was similar to that of cocaine). Among these was tropisetron. It proved to be a highly selective, potent 5-HT₃ receptor blocker, which was of particular value in dealing with nausea and vomiting caused by anticancer drugs such as cisplatin. This action was due to its ability to block presynaptic 5-HT₃ receptors in peripheral neurones which mediate the emetic reflex. Tropisetron was marketed in 1992. Granisetron is a similar drug developed at the Beecham Research Laboratories and introduced into the clinic in 1991.
Following the suggestion that the antimigraine action of the semi-synthetic alkaloid methysergide was due to its acting as a 5-HT agonist rather than as an antagonist, Glaxo researchers decided to develop a non-ergot 5-HT₁ agonist. They began by making analogues of 5-HT in which the hydroxyl group was replaced by other substituents. This resulted in compounds that, with one exception, were not only less potent but were also equiactive at 5-HT₂ receptors. However, activity at 5-HT₃ receptors was reduced. The sole compound that was twice as potent at 5-HT₁ receptors was also only one-twenty-fifth as potent at 5-HT₂ receptors. This was 5-carboxamidotryptamine (5-CT). When tested in a dog to confirm that it constricted the carotid bed, it was found to do the opposite in causing vasodilation and a drop in blood pressure. The conclusion was drawn that it was now also acting on a new type of receptor that was then designated as the 5-HT₇ receptor. Alkylating 5-CT resulted in analogues that were more active at the 5-HT₁ receptor than at the 5-HT₇ one. Further modification led to AH 25086, a compound that was highly selective for the HT₁ receptor and which produced constriction of the canine carotid bed. Following extensive testing, AH 25086 was found to relieve migraine in the clinic. Unfortunately, it was inactivated in the liver after being absorbed from the gut after oral administration. This was avoided by making the N-dimethyl derivative at the same time as replacing the amide side chain with a sulfonamide, as this had already been found to be just as effective. The resulting compound, sumatriptan, was one-fifth as active as 5-HT, but it was highly selective. It acts on the carotid arteries, which are stretched by an increased blood flow during a migraine attack, reducing dilation of these vessels. Although it and other triptans should be avoided in patients with cardiac disease, sumatriptan represented a major advance in the treatment of migraine.
A number of other orally active triptans have been introduced, including naratriptan, which has a longer lasting action, zolmitriptan, which has a faster onset of action, and also eletriptan, rizatriptan and frovatriptan.

**AMINO ACID ANALOGUES**

Gustav Stein and Karl Pfister at the Merck Sharpe and Dohme laboratories in Rahway, New Jersey, synthesised methyldopa as an analogue of the amino acid phenylalanine in 1953. It was found by Ted Sourkes at McGill University to be an in vitro inhibitor of the enzyme dopa decarboxylase, which plays a major role in catecholamine biosynthesis. In 1960, it was discovered that methyldopa was capable of reducing blood pressure and that its pharmacological profile justified a clinical trial. Since then, its main use has been in controlling raised blood pressure during pregnancy. Initially, it was assumed that its action was attributable to its effects on the peripheral synthesis of catecholamines, but subsequent studies revealed that it was due to a direct effect on the brain.

Once its ability to reduce blood pressure was recognised, Merck chemists synthesised analogues of methyldopa as potential hypotensive agents. Carbidopa was found to inhibit peripheral conversion of levodopa to dopamine, but it had no action within the central nervous system since it could not pass the blood–brain barrier. By good fortune the first studies on levodopa were then being conducted. It was realised that carbidopa opened up the possibility of using much lower doses of levodopa and reducing the side effects caused by the actions of dopamine outside the central nervous system. Carbidopa was subsequently marketed in a combined formulation with levodopa. Entacapone is a similar drug introduced in the 1990s.

**GABA analogues**

Baclofen is an analogue of GABA that was synthesised by Ciba chemists soon after GABA was shown to have a role in the central nervous system. It is a specific agonist that binds to spinal GABAg receptors (a subtype of the GABA receptors) to relax skeletal muscles, making it a useful antispastic agent in multiple sclerosis or spinal disease. As it is a GABA analogue, higher doses exert a sedating effect.

Gabapentin was designed by Parke, Davis and Company researchers as a GABA analogue that would readily enter the brain. Examination of its three-dimensional structure, however,
shows that the spatial positioning of its hydroxyl and amino groups does not correspond with those in GABA and it is now thought to act by binding to a brain protein.\textsuperscript{153} It was introduced in the United Kingdom in 1993, licensed for use in conjunction with other anticonvulsants for the management of partial epilepsy.

Vigabatrin\textsuperscript{154} is an analogue of GABA that chemically reacts with GABA transaminase to irreversibly inhibit this enzyme, which normally degrades GABA. It is given to patients with partial epilepsy who do not respond to other drugs.\textsuperscript{155}

PROSTAGLANDIN ANALOGUES

Being aware that removal of the metabolically sensitive 15-hydroxyl group of alprostadil resulted in loss of pharmacological activity, Paul Collins and his co-workers at G.D. Searle examined the effect of placing this group on the 16-position. The resulting compound had similar antisecretory activity to alprostadil, but was just as short-acting and feebly active by mouth since it was still a substrate for the dehydrogenase enzyme that deactivated natural prostaglandins. After it was discovered that enzymatic oxidation of the 15-hydroxyl group in the side chain of alprostadil could be sterically hindered by placing either one methyl group on the C-15 atom or two methyl groups on the adjacent C-16 atom, Collins placed a methyl group in his active analogue to determine whether it would block enzymatic deactivation. The resulting compound, prepared as the methyl ester, was misoprostol.\textsuperscript{156} When administered intravenously, it inhibited gastric acid secretion and was thirty-five times as potent as the analogue without the 16-methyl group. Misoprostol was also orally active and had a greatly increased duration of action. Subsequent studies confirmed that the active compound was the free carboxylic acid.

In 1975, André Robert reported that prostaglandins administered to rats concomitantly with indometacin could prevent the intestinal damage caused by indometacin on its own.\textsuperscript{157} He suggested that the administered prostaglandins provide mucosal protection and overcome the effects of the indometacin on internal prostaglandin biosynthesis. Consequently, misoprostol was introduced to prevent gastrointestinal ulcers in patients receiving prolonged treatment with indometacin and other non-steroidal anti-inflammatory drugs.

The placing of a second methyl group on the C-16 atom of alprostadil by researchers at the Ono Pharmaceutical Company in Osaka, Japan, provided a longer-acting prostaglandin, namely gemeprost.\textsuperscript{158} By administering it in a pessary to ripen and soften the cervix before abortion is induced, the problems associated with lack of specificity of this prostaglandin analogue are deftly avoided.
The brief duration of action of dinoprost was overcome by Upjohn chemists in 1971 by placing a methyl group on the 15-position to prevent enzymatic oxidation of the hydroxyl group. The resulting compound, carboprost, is administered to stop post-partum bleeding that has not ceased after ergometrine and oxytocin administration.

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