Vitamins

During the siege of Paris in 1871 the eminent French chemist Jean Baptiste Dumas published his observations on the disastrous consequences of feeding starving infants on artificial milk prepared by emulsifying fat in a sweetened, albuminous solution. This led to concern about dietary requirements, particularly with regard to mineral content. One of the leading investigators at that time was Gustav von Bunge at the University of Dorpat. Being particularly interested in the role of alkaline salts in the diet, he set his student Nicholas Lunin the task of establishing the effect of a sodium carbonate supplement on mice maintained on an artificial diet consisting of pure protein from milk (casein) and cane sugar. The mice receiving sodium carbonate survived from 12 to 30 days, in contrast to the 11 to 21 days of those denied the supplement. In an attempt to keep his mice alive longer, Lunin fed the mice on casein, milk fat, milk sugar (lactose) and salts, each of the ingredients being purified and then incorporated in the same proportion as in natural milk. This artificial milk did not improve survival rates, yet the mice thrived on powdered natural milk. This led Lunin to conclude that milk must contain unknown substances essential for growth and the maintenance of good health. Soon after publishing his results, Lunin left Dorpat to take up a clinical appointment at St Petersburg. Although his findings had presented a direct challenge to generally accepted views, he did not pursue them any further. Consequently, they were overlooked for many years. A similar fate befell a study by Cornelis Pekelharing of the University of Utrecht in 1905 as it was published in Dutch. This showed that mice could be kept healthy on an artificial diet to which had been added a small amount of whey (obtained by removal of fat and casein from milk).

Scant attention was paid to the results of feeding animals on artificial diets until 1906 when Gowland Hopkins of the University of Cambridge initiated his classical experiments on feeding rats with purified diets, with and without supplements of natural foods. These studies not only confirmed the findings of the earlier workers but also revealed that minute amounts of unknown substances present in normal food were essential for healthy nutrition. Hopkins kept the rats in good health on a diet consisting of casein, fat, starch, sugar and inorganic salts with a little milk added. Carefully controlled experiments showed that enough milk to increase the amount of solids in the diet by as little as 1% was quite sufficient. When Hopkins published his results in 1912, after a delay of five years caused by his ill health, his eminence in the scientific community ensured that the significance of the unknown accessory factors in food would never again be overlooked. By then, other work casting light on diseases caused through dietary deficiencies had given added significance to his conclusions.

WATER-SOLUBLE VITAMINS

Concerned at the high mortality from beriberi among soldiers living in barracks in Sumatra and prisoners in Java, the Dutch government in 1886 sent the bacteriologist Cornelis Pekelharing and a neurologist, Cornelis Winkler, to Batavia (now Djakarta) in the Dutch East Indies to investigate the cause of the malady. Beriberi was a progressive disease that manifested itself by peripheral neuritis, emaciation, paralysis and then cardiac failure. Its
incidence had greatly increased since 1870, especially among those living in barracks and prisons for more than three months. Two views prevailed as to its aetiology, namely that it was either of bacterial origin or else caused by a toxin in the rice that formed the staple diet of all those who were afflicted. That rice diets were the key to understanding the cause of the disease should have been evident from the study conducted in 1873 by van Leent, a Dutch naval surgeon who traced the high mortality from beriberi among Indian crews to their rice diet.\textsuperscript{4} Simply by putting them on the same diet as their European shipmates, he achieved a dramatic reduction in the morbidity of the disease.

Pekelharing and Winkler tentatively concluded that beriberi was of bacterial origin, and they recommended the disinfection of barracks and prisons. On returning to The Netherlands they left behind their assistant, Christiaan Eijkman, to isolate the causative organism. He was given a small laboratory in the Dutch military hospital in Batavia where he spent several months studying inoculation of fowls with body fluids from beriberi victims. Although his early studies were inconclusive, after a few months some of the fowls began to stagger around as if intoxicated. Eijkman recognised this as being due to polyneuritis (nerve degeneration) comparable to that seen in humans suffering from beriberi. Surprisingly, it was not confined to fowls that he had inoculated. Six months later, the fowl disease disappeared as mysteriously as it had begun. At this point, Eijkman realised that the diet of the fowls had changed shortly before they became diseased. When he had begun his investigations, the birds were fed on cheap rice. Later, Eijkman had been able to acquire supplies of the more expensive milled, polished rice fed to patients in the military hospital. When a new director of the hospital was appointed, Eijkman was told to cease this practice. It was the change back to unmilled rice that led to the disappearance of the disease in the fowls.

Eijkman now carried out an experiment which involved feeding milled and unmilled rice to two groups of fowls. The results proved conclusively that milling removed some unknown ingredient that prevented polyneuritis, present in the germ or pericarp. Nevertheless, Eijkman was not yet convinced that this simple explanation could account for the occurrence of beriberi in humans. Further investigations were required before this conclusion could be drawn, but he was unwilling to experiment upon humans. Meanwhile, he published his findings in the same obscure Dutch East Indies journal as had van Leent.\textsuperscript{5} It was another seven years before he presented a short account in a prominent German journal in 1897.\textsuperscript{6} Eijkman asked his friend Adolphe Vorderman, the inspector of health in Java, to ascertain the frequency of beriberi in the 101 prisons on the island, as well as the type of rice being consumed. Vorderman discovered that in the years 1895–1896, in those prisons where the inmates prepared their own crude rice, the disease was rarely seen. However, in prisons where machine-milled, polished rice was supplied there was a high incidence of beriberi. It was even found that the degree of milling influenced the incidence of disease. Eijkman then showed that the outer layers of rice, the silver-skin, could prevent polyneuritis in fowls. In 1897, Vorderman demonstrated in a prison in the village of Tulung Agung that beriberi could be eliminated by replacing polished rice with unmilled rice.

When Eijkman returned to The Netherlands in 1896 to take up a post at the University of Utrecht, he was convinced that beriberi was caused by gut microbes converting some constituent of the carbohydrate-rich rice into a toxic substance that was then absorbed into the circulation. An antidote to this was presumed to be present in the outer layers of the rice that were removed during milling.

Gerrit Grijns, who was Eijkman’s successor at Batavia, showed that polyneuritis could be produced in fowls by feeding them on unmilled rice that had been autoclaved for two hours at 120 °C. Unmilled rice that had not been exposed to this destructive treatment consistently protected the birds against the disease. Grijns also found that feeding fowls on vegetables known to prevent beriberi protected them against the occurrence of polyneuritis. By means of these and other experiments, he finally came to the conclusion that in order
to maintain the functional integrity of their nervous system it was essential for the diets of animals and humans to contain a protective substance that was present in unmilled rice, meat and vegetables. If the diet was deficient in this protective substance, beriberi resulted.

**Thiamine**

In 1906, Eijkman established that the protective substance was soluble in water and had a low molecular weight. Several unsuccessful attempts were made to isolate it in crystalline form. In one by Casimir Funk, a Polish scientist working in the Lister Institute in London, a crystalline compound extracted from rice polishings cured experimentally induced polyneuritis in pigeons. Subsequently, it was established that these crystals had probably consisted of nicotinic acid contaminated with the true antineuritic substance. Believing he had isolated the genuine protective factor and that it was an amine, Funk proposed that it be called ‘beriberi vitamine’. His new term was altered to ‘vitamin’ by his colleague Jack Drummond in 1920, after it was realised that none of the protective substances then isolated were amines. Drummond proposed that different vitamins be distinguished by the use of letters of the alphabet, the antineuritic vitamin being described as vitamin B.

A reliable method of detecting the antineuritic substance was devised by Barend Jansen, who was based in a new laboratory in Batavia, built for the Dutch East Indies Medical Service. With considerable persistence, he established that small birds known as ‘bondols’ (Munia maja) were much more susceptible to polyneuritis than were fowls. When fed on polished rice for only ten days, they developed polyneuritis, instantly detectable by their flying in characteristic circles. By 1920, Jansen had perfected a reliable assay which involved measuring the ability of his rice extracts to prevent, rather than cure, polyneuritis induced by feeding the bondols on polished rice. Due to its instability, years of patient work were required before Jansen and Donath finally isolated crystals of the pure vitamin in 1926. Samples of the crystalline vitamin were sent to Eijkman at Utrecht, where he demonstrated that the addition of 2–4 mg of these to every kilogram of polished rice restored its full antineuritic value. Three years later, Eijkman and Hopkins were jointly awarded the Nobel Prize for Physiology and Medicine for their work on vitamins.

The Jansen–Donath extraction process was too expensive for commercial exploitation. In 1933, Robert Williams developed a new isolation process for the antineuritic vitamin, by then known as vitamin B₁. His work was carried out privately in his spare time while he was the chemical director of the Bell Telephone Laboratories in the United States, but had spent more than 20 years researching the vitamin while at the Bureau of Chemistry set up in the Philippines by the US Army Medical Commission for the study of tropical disease. Williams contacted Ralph Major, director of research at the Merck Laboratories in Rahway, with the outcome that collaboration with the company began at once. Early in 1936, Williams presented his proposal for the chemical structure of the vitamin. Shortly after, he and Joseph Cline of Merck published a preliminary account of their synthesis which confirmed the validity of the proposed structure. Immediately after this appeared in print, Williams learned that Hans Andersag and Kurt Westphal at the Elberfeld laboratories of I.G. Farben had synthesised the vitamin some months earlier. It was also synthesised around this time by Alexander Todd and Franz Bergel at the Lister Institute in London. In 1937, Williams licensed Merck to produce the vitamin commercially by his synthetic process. Much of the profit from the Williams patents was devoted to a fund that supported nutritional research.
In 1937, several European countries accepted Jansen’s proposal that the anti-berberi vitamin should be known henceforth as ‘aneurine’. The US Council on Pharmacy and Chemistry rejected this since their policy was to avoid drug names with any therapeutic connotation. Williams suggested that it be called thiamine, a term that highlighted the presence of the sulfur atom. This was not universally accepted until the International Union of Pure and Applied Chemistry’s Commission on the Nomenclature of Biological Chemistry approved the name in 1951.

Riboflavine

Until 1919, it was generally believed that there were only two water-soluble vitamins, namely vitamins B and C. Elmer McCollum at the University of Wisconsin had discovered the existence of a fat-soluble vitamin in 1913 while feeding rats on artificial diets. Continuing his nutritional studies, he then found that if the commercially supplied lactose (milk sugar) that he had been using was purified by recrystallisation, the rats exhibited manifest evidence of a growth disorder due to a dietary deficiency that he could correct merely by supplementing the diet with the aqueous mother liquor from which the lactose had been crystallised. This led him to conclude that a water-soluble vitamin existed, whereupon he then learned of the earlier studies by Eijkman and Grijns. After repeating their work with polished rice, he came to the conclusion that the antineuritic vitamin and his rat growth-promoting vitamin were identical.

In a review article that appeared in the *Journal of Biological Chemistry* in 1919, H.H. Mitchell of the University of Illinois questioned the assertion that the antineuritic vitamin and the rat growth vitamin were identical. He claimed that all the evidence was circumstantial. During the next few years, experimental results tended to support Mitchell’s contention and researchers began to speak of the vitamin B complex.

In 1927, the British Committee on Accessory Food Factors distinguished between vitamin B₁, the antineuritic vitamin isolated the previous year, and the more heat-stable vitamin B₂. The following year, Harriet Chick and M.H. Roscoe of the Lister Institute developed an assay for vitamin B₂ activity. This involved measuring the effect of test material on young rats fed on a diet deficient in the vitamin B complex, but to which vitamin B₁ had been added. In the absence of any vitamin B₂ supplementation, the rats exhibited loss of hair from the eyelids, sealing of the eyelids by a sticky exudate, dermatitis, blood-stained urine and stunted growth. Each assay took three or four weeks to complete. Tedious as this must have been, it was sufficient to encourage Richard Kuhn of the Institute of Chemistry in the University of Heidelberg and Theodore Wagner-Jauregg of the Kaiser Wilhelm Institute for Medical Research to join with the paediatrician Paul György, an émigré Hungarian, in his attempt to isolate pure vitamin B₂. Wagner-Jauregg noticed that all extracts that proved active by this assay procedure exhibited an intense yellow–green fluorescence, the intensity of which was proportional to potency. When attempts were first made to isolate the fluorescent material, the growth-promoting activity of the extracts deteriorated. It was then realised that other growth-promoting vitamins must have been present prior to refinement of the crude vitamin B₂, thus pointing the way to the discovery of further members of the vitamin B complex. The biological assay procedure had to be modified to allow for this, with the outcome that the yellow–green fluorescent material was isolated from spinach, kidney and liver, proving identical in each
case. The vitamin was crystallised at Heidelberg in 1933 and named ‘riboflavine’\(^{18}\) (American workers at one time referred to it as vitamin G; confusingly, the term vitamin B\(_2\) has been retained despite the fact that this was originally applied to crude preparations containing several members of the vitamin B complex).

Two years later, Richard Kuhn at Heidelberg\(^{19}\) and Paul Karrer at the University of Zurich\(^{20}\) almost simultaneously synthesised the vitamin. The latter’s process was adapted by Hoffmann-La Roche for commercial production. In 1937, Karrer was awarded a Nobel Prize for Chemistry, shared with Norman Haworth for their work on vitamins. The following year, Kuhn was similarly honoured, but the Nazi government in Germany made him decline the award.

Nicotinamide

When the distinction between vitamin B\(_1\) and the crude vitamin B\(_2\) had first been made, it was generally assumed that the latter was the pellagra-preventing vitamin (P-P factor). The Spaniard Gaspar Casal described pellagra in the early eighteenth century and attributed it to consumption of diets rich in maize.\(^{21}\) The disease was given its name in 1771 by the Italian physician Francesco Frapolli on account of the characteristic skin changes (\(pelle = \text{skin}, \ agra = \text{dry}\)) that it caused.\(^{22}\) These were in addition to gastrointestinal disturbances and degenerative changes in the central nervous system that ultimately lead to insanity.

It was not until an epidemic swept through the southern United States in the early years of the twentieth century that the cause of pellagra was subjected to experimental scrutiny. An extensive series of clinical and epidemiological studies was initiated in 1914 by a team from the US Public Health Service, led by Joseph Goldberger. The initial conclusion was that diets rich in maize were to blame, this being consistent with the earlier demonstration by Edith Willcock and Hopkins that young mice failed to grow on diets in which zein from maize was the sole source of protein, zein being deficient in tryptophan.\(^{23}\) However, in 1920, Carl Voegtlin and his colleagues from the pharmacology division of the Public Health Service discovered that pellagra could be cured by administration of dried yeast or aqueous extracts of yeast, these preparations being known to be a rich source of vitamin B.\(^{24}\) Further experiments indicated that the active material in the yeast was not destroyed by heating the yeast at 52 °C. Since Voegtlin had previously shown that vitamin B\(_1\) had no beneficial value in pellagra, Goldberger and his colleagues concluded that vitamin B\(_2\) must be the P-P factor.\(^{25}\)

After vitamin B\(_2\) was found to be a complex mixture and riboflavine to have no P-P activity, the search for the true P-P factor was intensified. It was greatly facilitated through the earlier recognition by T.N. Spencer, a veterinarian from Concord in North Carolina, that a disease in
dogs known as ‘black tongue’ was the canine counterpart of human pellagra. Goldberger then
developed an assay for the P-P factor based on prevention of ‘black tongue’ in dogs. He was
able to demonstrate that liver was one of the richest sources of the P-P factor. Conrad
Elvehjem and his associates in the Department of Agricultural Chemistry at the University of
Wisconsin-Madison finally isolated the P-P factor from fresh liver in 1937.26 The vitamin was
immediately recognised to be nicotinamide (niacinamide), a substance already being studied
by biochemists. With Wayne Woolley, Elvehjem demonstrated that both nicotinamide and
nicotinic acid (niacin) were capable of preventing and curing ‘black tongue’ in dogs.27 Human
trials followed at once, which proved highly successful. Since nicotinic acid had been
synthesised by Albert Ladenburg 40 years earlier, there was no problem in producing large
amounts for the treatment of pellagra. The amide was readily prepared from the acid.

Rudolf Altschul and Abram Hoffer at the University of Saskatchewan discovered in 1955
that high doses of nicotinic acid lowered serum cholesterol levels in humans.28 It was the first
drug ever used for this purpose, but vasodilation was a dose-limiting side effect.29 The
vasodilatory activity, however, has been exploited in remedies for chilblains and in the
formulation of counter-irritant creams.

Pyridoxine

The isolation of further members of the vitamin B complex rapidly followed that of
nicotinamide. The first of these was discovered as a consequence of studies on young animals
deliberately deprived of the B group of vitamins other than those already known. The main
difficulty facing the researchers was that of unravelling the complex pathological changes
arising from deficiency of unidentified vitamins. It was while working at Cambridge
University in 1934 that Paul György suggested that one such unidentified vitamin could
protect rats from a specific type of skin lesion. He proposed the name vitamin B₆ for this rat
antidermatitis factor, which he and T.W. Birch did much to characterise chemically. Early in
1938, Samuel Lepkovsky30 of the College of Agriculture at the University of California,
Berkeley, informed György that he and John Keresztesy31 of Merck and Company were each
independently about to submit papers describing the crystallisation of the vitamin. This
magnanimous gesture enabled György32, now at Western Reserve University in Cleveland, to
publish his own account of the crystallisation shortly after.

Within a year of its isolation, both Karl Folkers33 of Merck and Company and also Kuhn34
at Heidelberg had established the chemical structure of vitamin B₆ and had then synthesised it.
György proposed that it henceforth be known as ‘pyridoxine’. It should, in passing, be
mentioned that following American government indications that it favoured the supplemen-
tation of foods and cereals with vitamins, Merck and Company had invested heavily in
equipment to separate the B vitamins from natural sources such as yeast. The success of their own and rival chemists rapidly rendered this obsolete.

**Pantothenic Acid**

In 1939, Thomas Jukes, a colleague of Lepkovsky at the University of California, and also Woolley, Waisman and Elvehjem at the University of Wisconsin, simultaneously discovered that pantothenic acid was the hitherto unidentified vitamin whose deficiency had been shown to cause dermatitis in chickens. This had been isolated the previous year by Roger Williams at the University of Oregon during investigations into nutrients essential for the growth and replication of cultured yeast cells. It had taken him four years to isolate and purify this new yeast growth factor after differentiating traces of it from other essential nutrients present in food extracts. It was synthesised by Merck chemists in 1940.

![Pantothenic acid](image)

**Biotin**

Roger Williams’ pioneering studies on yeast growth factors had begun in 1919 when he tried unsuccessfully to develop a new type of assay for vitamin B_1_ activity. Nevertheless, his work stimulated microbiologists to isolate growth factors for other organisms, including bacteria. In 1931, a Canadian scientist, W.L. Miller, detected the presence of two yeast growth factors in malt, namely Bios I and II. He identified the former as inositol, a sugar long known to be present in muscle. Five years later, Kögl and Tönnis isolated crystals believed to be Bios II from boiled duck egg yolks. They named these ‘biotin’.  

![Biotin](image)

Vincent Du Vigneaud and Donald Melville determined the chemical structure of biotin in 1942, which was synthesised a year later by Karl Folkers and his colleagues at Merck.

**Cyanocobalamin**

The discovery of cyanocobalamin (vitamin B_12_) came about as a consequence of fundamental studies into bile pigment metabolism and its relation to liver disease by George Whipple that began in 1914 at the University of California Medical School in San Francisco. He found it necessary to extend his investigations to cover the rate of formation of haemoglobin, the pigment in red blood cells from which the bile pigments are derived. He did this simply by draining blood from dogs and waiting to see how long it took for haemoglobin levels to return to their original level. When this unexpectedly revealed that diet influenced the rate of haemoglobin regeneration, Whipple’s interest in liver disease led him to examine the effect of feeding liver to his anaemic dogs. It proved to have a more powerful effect than any other food. On taking up a new appointment at the University of Rochester, New York, Whipple refined his techniques to confirm the earlier studies. His results came to the attention of
George Minot at Harvard, a clinician who had been investigating the influence of diet on patients with pernicious anaemia, since some of its symptoms resembled those of beriberi and pellagra. Pernicious anaemia was at that time an incurable disease characterised by failure of normal red cell formation, with death within a few years.

When Minot fed liver to a few patients with pernicious anaemia their condition improved, but the results were hardly conclusive. A detailed investigation followed, with 45 patients receiving enormous daily doses of liver by mouth. When the trial was completed in May 1926 the results were startling. Many patients showed obvious signs of improvement within a week, their red blood cell count being restored to satisfactory levels within two months. For this outstanding contribution, Whipple, Minot and Murphy received the Nobel Prize for Medicine and Physiology in 1934.

Eating as much as half a kilogram of liver each day was a daunting prospect for anyone, let alone a sick patient. To overcome this, Edwin Cohn at the Harvard Medical School prepared an extract that was marketed by Eli Lilly and Company in 1928. Two years later, Lederle Laboratories introduced a more refined extract for intramuscular injection. This was far more satisfactory since the real cause of pernicious anaemia was a defect in gut absorption processes. A single injection once every one to three weeks proved adequate.

During the early 1940s it was realised that the loss of activity when liver extracts were decolorised with charcoal was due to adsorption of the active material. This was ultimately turned to advantage when it was shown that under certain conditions the active material could be eluted from charcoal to give a much purer product. This paved the way for eventual isolation of crystals of the active principle in 1948 by Lester Smith of Glaxo Laboratories in the United Kingdom and also by Karl Folker and his colleagues at Merck and Company in the United States. Later in the year, the Merck researchers isolated the vitamin from a strain of Streptomyces griseus used in streptomycin production. This meant that a cheap source had been found and commercial production began in 1949.

![Cyanocobalamin](image)
As the vitamin turned out to be a cobalt-containing molecule it received the approved name of cyanocobalamin, although it became widely known as vitamin B₁₂. Its chemical structure was elucidated in 1955 through the collaboration of chemists from the University of Cambridge, led by Alexander Todd, with X-ray crystallographers from the University of Oxford, led by Dorothy Hodgkin, and a team from Glaxo, led by Lester Smith. Todd was awarded the Nobel Prize for Chemistry in 1957 and Hodgkin in 1964.

**Folic Acid**

In 1930, Lucy Wills and S.N. Talpade of the Haffkine Institute in Bombay found that undernourished mothers of premature babies were consuming diets deficient in the vitamin B complex. They thought this might account for the manifestation of pernicious anaemia-like symptoms during pregnancy. Wills went on to study textile workers in Bombay who had developed a form of anaemia resembling pernicious anaemia except for the absence of neurological complications. She described this as ‘tropical macrocytic anaemia’ because of the presence of many large, immature blood cells. In contrast to pernicious anaemia, this macrocytic anaemia responded positively to treatment with a proprietary brand of yeast extract (Marmite) that was rich in the vitamin B complex. When monkeys were fed on diets similar to those eaten by the anaemic patients, they also developed the disease. Administration of yeast extract or liver also cured the monkeys, but injections of liver extract normally used in treating pernicious anaemia proved worthless both in monkeys and humans suffering from the macrocytic anaemia. Evidently, the purification of the liver extract had removed a protective factor that was different from vitamin B₁₂.

The significance of Wills’ results was not appreciated. However, in 1935 similar observations on monkeys were noted by Paul Day of Little Rock University, Arkansas, in the course of feeding experiments designed to produce cataracts from riboflavine deficiency. He blamed a dietary deficiency when his monkeys developed anaemia and died from complications. After Day had managed to correct the purported deficiency with either yeast supplements or whole livers, he proposed that the protective factor be called vitamin M. In the absence of a convenient assay system using small animals with a short lifespan, Day was unable to consider the isolation of the vitamin.

In 1939, Albert Hogan and Ernest Parrott at the University of Missouri found that chickens fed on a simple diet sometimes became anaemic and failed to grow. Abnormalities in their red blood cells were traced to variations in the quality of the commercial liver extract incorporated in the feedstuff. The evidence pointed to deficiency of an unidentified B complex vitamin that they described as vitamin B₉. Unlike Wills and Day, Hogan was able to conduct assays for vitamin activity and so proceed with its isolation from liver. In the autumn of 1940 he approached Parke, Davis and Company, who put a team of scientists on to the project. It took two and a half years before crystals of the anti-anaemic factor were isolated. It turned out to be an acid. In the interim, events had moved rapidly.

In attempting to devise artificial media that would permit determination of the exact nutritional requirements of bacteria such as *Lactobacillus casei*, Esmond Snell and William Peterson at the University of Wisconsin found little bacterial growth with a hydrolysed casein-based culture medium, unless plant or animal extracts were incorporated. Further investigation revealed that yeast extract was the richest source of growth-promoting material, and in 1939 an active fraction was separated from this source by means of elution chromatography. Peterson, assisted by Brian Hutchings and Nestor Bohonos, went on to obtain a *Streptococcus lactis* growth-stimulating fraction from liver. Snell transferred from Wisconsin to work with Roger Williams, now at the University of Texas, where, with the assistance of Herschel Mitchell, they isolated from spinach a concentrate of a *Lactobacillus casei* growth factor that they named folic acid (Latin: *folium* = leaf). Elvehjem and Hart at
Wisconsin then found it to be capable of preventing anaemia in chickens.\textsuperscript{60} It seemed that it must be the same as Hogan's anti-anaemic factor, vitamin B\textsubscript{c}, as the physical properties of the two substances were similar. Hogan confirmed that the substances had identical biological properties.

In 1938, Robert Stockstad and P.D.V. Manning of the Californian-based Western Condensing Company were involved in formulating a diet that would be suitable for assaying riboflavin on chickens when they came to the conclusion that an unknown dietary growth factor existed.\textsuperscript{61} Tentatively, they described it as the U factor and stated that it was present in certain yeast extracts.\textsuperscript{62}

In 1941, Stockstad was recruited by Lederle Laboratories to work on liver extracts at their Pearl River research centre. Two years later, he isolated crystals of the \textit{L. casei} growth factor from 1.5 tons of liver.\textsuperscript{63} These proved to be identical to the vitamin B\textsubscript{c} that had just been described by Hogan and the Parke, Davis and Company researchers. The structure was determined by Lederle researchers,\textsuperscript{64} who went on to achieve the total synthesis of folic acid in August 1945.\textsuperscript{65}

**Folinic Acid**

A growth factor required by \textit{Leuconostoc citrovorum}, known as either 'citrovorum factor', 'leucovorin' or 'folinic acid', was isolated\textsuperscript{66} and subsequently synthesised by Lederle chemists.\textsuperscript{67} It acts as an antagonist of antifolate drugs and has been used clinically to treat methotrexate toxicity. Such treatment is described as folic acid rescue.

Folinic acid enters human cells by the folate uptake pathway. Once inside the cells it is rapidly metabolised to 5-methylenetetrahydrofolate, an active form of folic acid that can participate in a one-carbon transfer system to convert uracil to thymine. Folinic acid is thus able to bypass the blocking of tetrahydrofolate formation caused by antifolates.

**Ascorbic Acid (Vitamin C)**

The investigations into the nature of the antineuritic vitamin were directly responsible for the discovery that absence from the diet of another water-soluble vitamin was the cause of scurvy. In 1536, the French explorer Jaques Cartier had vividly described the nature of this disease.
that afflicted all but ten of the 110 men aboard his three ships wintering in the frozen St Lawrence River. The victims' weakened limbs became swollen and discoloured, while their putrid gums bled profusely. The captain of his ship learned from an Indian how to cure the sailors with a decoction prepared from the leaves of an evergreen tree. Miraculously, so it seemed, the remedy proved successful. Nearly 30 years later, the Dutch physician Ronsseus advised that sailors consume oranges to prevent scurvy and in 1639 John Woodall, one of England's leading physicians, recommended lemon juice as an anti-scorbutic.  

Notwithstanding these earlier developments, it is the Scottish naval surgeon James Lind who is remembered as the first person to conduct a controlled clinical trial, through which he proved that scurvy could be cured by drinking lemon juice. In May 1747, he tested a variety of reputed remedies on 12 scurbutic sailors quartered in the sick bay of the fourth-class ship called the Salisbury. Two were restricted to a control diet, but each of the others were additionally given one of the substances under trial. The two seamen who were provided with two oranges and a lemon each day made a speedy recovery, one of them being fit for shipboard duties in only 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 those who had been given either oil of vitriol (dilute sulfuric acid), vinegar, sea-water or only the control diet. He drew the obvious conclusions, which were acted upon by Captain James Cook on his second voyage round the world. Although Cook was at sea for three years, none of his crew died from scurvy thanks to adequate provision of lemon juice, as well as fresh fruit and vegetables. Surprisingly, it was not until 1795 that the Admiralty finally agreed to Lind's demands for a regular issue of lemon juice on British ships. The effect of this action was dramatic; in 1780, there had been 1457 cases of scurvy admitted to Haslar Naval Hospital, but 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 that had been introduced had only about a quarter of the antiscorbutic activity of the lemon juice that it had widely displaced.

In 1899, Stian Erichsen wrote in Tidsskrift for den Norske Laegeforening (the Journal of the Norwegian Medical Association) that a mysterious illness afflicting sailors on very long voyages was caused by lack of fresh food. Concerned at the growing incidence of this disease, which had similarities to both beriberi and scurvy, the Norwegian Navy asked Axel Holst and Theodor Frolich of Christiania University in Oslo to investigate the matter. Holst visited Gerrit Grijns in Batavia before returning to carry out experiments on guinea pigs. Fortunately for him, guinea pigs are exceptionally sensitive to ascorbic acid deficiency, and so he and Frolich readily induced a condition analogous to human scurvy by feeding their animals on polished rice. This was not alleviated by giving the guinea pigs rice polishings, but fresh fruit or vegetables known to cure scurvy restored them to good health. On the basis of these findings, Holst argued, in 1907, that in addition to the antineuritic dietary protective substance postulated by Grijns there must also exist an antiscorbutic one, and the disease among Norwegian sailors could be prevented by appropriate dietary measures.

Holst and Frolich went on to demonstrate that the antiscorbutic factor was soluble in water. They showed that, like the antineuritic substance, it was of low molecular weight. They also found that when foods were subjected to drying, the antiscorbutic principle was destroyed. Their pioneering studies were confirmed by work on monkeys carried out at the Lister Institute during the First World War in the wake of outbreaks of scurvy among British troops serving in the Middle East; this was despite the provision of lime juice. Only then was it recognised that the juice of West Indian limes had poor antiscorbutic activity in comparison to that of lemons. In 1920, Jack Drummond proposed that the antiscorbutic protective substance be called vitamin C until its chemical structure was established.

Working at the Lister Institute, Sylvester Zilva began to prepare concentrated extracts of the vitamin in 1918. Five years later, he introduced a highly potent concentrate.
University of Pittsburgh, Charles King obtained a more stable form of this by removing traces of heavy metals that catalysed oxidation. In the autumn of 1931, after four years of intensive investigations, King finally isolated pure crystals of the vitamin from lemon juice. Tests with these showed that a daily dose of 0.5 mg could prevent a guinea pig becoming scorbatic on a diet deficient in the vitamin. The crystals turned out to be very similar to an acidic carbohydrate isolated in Gowland Hopkins’ laboratory at Cambridge in 1928 from adrenal glands, cabbages and oranges by Albert Szent-Györgyi, a Hungarian biochemist who had been awarded a Rockefeller Fellowship to investigate oxidation–reduction processes in the adrenals. The possibility that his new compound, then thought to be a hexuronic acid, might be the antiscorbutic vitamin had apparently been ruled out by results obtained by Zilva, but King’s successful isolation of the vitamin reopened the issue.

Assisted by Joseph Svirbely of King’s department, Szent-Györgyi found that 1 mg daily of his hexuronic acid protected guinea pigs against scurvy. King gained further confirmation of the identity of his vitamin with Szent-Györgyi’s acid. In order to establish the nature of the vitamin, Szent-Györgyi initiated a collaborative programme with the University of Birmingham, a leading centre in the field of carbohydrate chemistry. It soon became evident that the vitamin could not be a hexuronic acid, and Szent-Györgyi and Norman Haworth then proposed that it should be known as ascorbic acid.

In a letter appearing in the *Journal of the Society of Chemistry and Industry* on 10 March 1933, Edmund Hirst published the correct structure for ascorbic acid as determined by him and his colleagues at Birmingham. A race to synthesise the vitamin began, and on 11 July 1933 Tadeus Reichstein at the Eidgenossische Technische Hochschule (ETH) in Zurich submitted a letter to the editor of *Nature* giving a preliminary account of his successful synthesis of ascorbic acid. It did not appear in print until over five weeks later, by which time a preliminary account of a synthesis by Haworth and Hirst had already appeared in the *Journal of the Society of Chemistry and Industry* on 4 August 1933, having been submitted only three days earlier! Reichstein, however, amassed a fortune from patent royalties after Hoffmann–La Roche began commercial production of synthetic ascorbic acid in 1934. Szent-Györgyi received the Nobel Prize for Medicine and Physiology in 1937 for his work on the biochemical role of ascorbic acid, while Haworth shared the Nobel Prize for Chemistry.

**FAT-SOLUBLE VITAMINS**

In the course of examining the effect on rat growth of varying the mineral content of artificial diets, Elmer McCollum and his assistant Marguerite Davis at the University of Wisconsin noted that normal growth patterns could be maintained for only 70–120 days. However, when natural diets were reintroduced, normal growth was restored. Many experiments had to be carried out before suspicion fell on the nature of the fat content of the artificial diet. To confirm that a fat-soluble accessory factor was present in only certain foods, McCollum and Davis supplemented the deficient diet with ether extracts of foods containing fat. This proved that the factor was to be found in butterfat and egg yolk, but not in lard. When they reported their findings much surprise was engendered in nutritional circles as it had universally been believed that the role of fats in the diet was solely to produce energy, the qualitative differences between them being of no consequence. Later, when McCollum detected a water-soluble
accessory food factor in milk, he named the two factors he had discovered as ‘fat-soluble A’ and ‘water-soluble B’. These terms were changed in 1920 by Jack Drummond to vitamin A and B respectively, the latter being identical to the antineuritic vitamin first discovered by Eijkman.

Retinol (Vitamin A)

McCollum’s findings were immediately pursued at Yale University by two of the leading American nutritionalists, Thomas Osborne and Lafayette Mendel. They noticed that in animals fed on a diet deficient in the fat-soluble factor, a characteristic eye disease occurred. This had been observed in malnourished animals before, but had not been considered of any particular significance. Once the association with vitamin deficiency became evident, attitudes quickly changed as researchers realised that many clinical reports had associated eye disorders with nutritional factors. Of particular relevance was one published in 1904 by M. Mori, a Japanese ophthalmologist. This described an eye disease characterised by dryness of the conjunctiva (xerophthalmia) frequently seen among infants fed on cereals and beans, but never found among the children of fishermen.76 The author of the report stated that the disease was due to lack of fat in the diet and could rapidly be cured by administering cod liver oil. Osborne and Mendel were able to demonstrate that both butter fat and cod liver oil could alleviate the ophthalmic disorder in their experimental animals.77 Not long after, an outbreak of serious eye disease, sometimes blinding, occurred among Dutch children fed on fat-free skimmed milk because of wartime measures to ensure increased export of butter. These infants were cured with cod liver oil supplements and full cream milk. At Wisconsin, S. Mori subsequently carried out extensive microscopic studies on the eyes of rats prepared for him by McCollum, thereby elucidating the pathology of xerophthalmia.78 He found that the dryness of the eyes was due to changes (keratinisation) in the cells lining the tear glands. As a result, the tears were unable to exercise their protective role against bacteria, and infection of the inner surfaces of the eyelids ensued. In severe cases, the infection spread into the eye, causing ulceration of the cornea. The interest aroused by the discovery of the relationship between eye disease and vitamin A deficiency drew attention to old reports of night-blindness being cured by the eating of liver. Biochemists eventually established that the vitamin was converted to the pigment in the retina known as visual purple (rhodopsin).

In 1924, Jack Drummond at University College London developed a steam distillation process to separate vitamin A from other unchanged fats remaining in cod liver oil after boiling in alcoholic potassium hydroxide (to saponify biologically inactive fats). The following year, he and Otto Rosenheim exploited their discovery that isolation of the vitamin could be greatly facilitated by measuring the intensity of the purplish colour it produced on reacting with arsenic trichloride. In collaboration with Isidor Heilbron at the University of Liverpool, Drummond made further improvements by developing a high-vacuum distillation technique that ultimately yielded almost pure vitamin.79,80 In 1929, after it was discovered that livers of other types of fish were often richer sources of vitamin A than cod liver, Abbott Laboratories and Parke, Davis and Company jointly began to process halibut liver oil for its vitamin content. The resulting product, though of high potency, was not particularly palatable on account of its strong fishy smell.
In 1931, Paul Karrer at Zurich University introduced adsorption chromatography to isolate a viscous yellow oil consisting of almost pure vitamin A. With this, he determined the chemical structure, reporting it two years later. However, it was not until 1937 that pure vitamin A, retinol, was crystallised by Harry Holmes and Ruth Corbet of Oberlin College, Pennsylvania, using fractional freezing and cold filtration. In 1947, Otto Isler of Hoffmann–La Roche introduced a commercial synthesis of the vitamin, as a consequence of which fish liver oil extraction processes are no longer in use.

As long ago as 1925 it was observed that rats fed on a vitamin A-deficient diet developed dyskeratotic skin conditions. This finding was not exploited until 1959 when the Berlin dermatologist Gunter Stütten showed that retinol palmitate inhibited the growth of benzpyrene-induced tumours in mice when administered systemically, but not if applied topically – even though it penetrated the stratum corneum of the skin. When the same thing happened on treating various dyskeratoses, Stütten came to the conclusion that retinol was only effective after it had undergone metabolic activation. He then collaborated with Hoffmann–La Roche to arrange a study with the major metabolite of retinol, which is now known as ‘tretinoin’. This showed it to be effective when applied topically in a variety of skin conditions. Unfortunately, healing was preceded by local irritation, which militated against clinical acceptance of the drug. However, it was reported in 1969 that tretinoin did not cause irritation when used to treat acne vulgaris. Consequently, this and treatment of photodamaged skin became its principal clinical application. It is now also given by mouth in acute promyelocytic leukaemia, resulting in three out every four patients remaining disease free after five years. The mechanism of action is unknown, but its administration restores the ability of defective granulocyte precursors in the bone marrow to develop normally.

Isotretinoin, the synthetic geometric isomer of tretinoin, was found to be just as effective in the treatment of acne, but when given by mouth it had a greater safety margin. Unfortunately, it is teratogenic and so cannot be prescribed for women of child-bearing age unless effective contraceptive cover is provided.

**Vitamin D₂ (Ergocalciferol, Calciferol)**

In 1912, Gowland Hopkins suggested that rickets might be yet another of the diseases caused by deficiency of an accessory food factor. Outwardly, rickets (rachitis) was characterised by
deformity of the limbs of infants arising from failure of calcium phosphate to be deposited at the growing ends of their bones. Unchecked, the disease not infrequently involved the central nervous system, which could be fatal. Although known for centuries, rickets reached epidemic proportions early in the twentieth century in the industrial cities of Northern Europe and America. This spurred Hopkins to recommend to the newly formed Medical Research Committee that it should designate rickets as a subject for special study. He recommended research should be undertaken by one of his former students, Edward Mellanby. The Committee agreed and Mellanby began work in 1914. Travelling between London and Cambridge, where he had access to a colony of puppies, he painstakingly conducted hundreds of feeding experiments in an attempt to identify the type of diet that induced rickets. In 1918, he was able to inform the Physiological Society that he could produce rickets in puppies by feeding them for three or four months on either a diet of milk, rice, oatmeal and salt, or on milk and bread. By adding a variety of foods to these rachitic diets, Mellanby was able to confirm that animal fats such as butter, suet and cod liver oil had antirachitic activity. The latter was a Northern European folk remedy that became esteemed as a tonic in the late eighteenth century, since when it had been widely employed in the palliation of debilitating diseases such as tuberculosis and rheumatism. The Parisian physician Armand Trousseau referred to its use for treating rickets in his *Clinique Médicale de l’Hôtel-Dieu de Paris*, published in 1861. However, it was not until after Mellanby had offered experimental proof of the value of cod liver oil that any significant reduction in the incidence of the disease was recorded. By the early 1930s the disease was no longer seen in London.

Mellanby believed that the antirachitic vitamin and vitamin A were identical, although he recognised that the evidence was not altogether conclusive. In an attempt to settle the issue, he took advantage of Hopkins’ new observation that vitamin A activity of hot butterfat was destroyed by bubbling oxygen through it. When Mellanby treated both butterfat and cod liver oil in this manner, he found the latter retained antirachitic activity. He was undecided as to whether this proved the existence of a second fat-soluble vitamin or merely reflected the presence either of a larger initial amount of vitamin A in the cod liver oil or else of an antioxidant. McCollum, who had moved from Wisconsin to Johns Hopkins University, set out to settle the matter by experimenting on rats he had already made rachitic by feeding them on artificial diets containing an unfavourable balance of calcium and phosphorus. He heated cod liver oil in a current of air for a prolonged period to ensure oxidation of all the vitamin A present and then demonstrated that the oil still retained its protective antirachitic action. His results were published in 1922. They conclusively proved the non-identity of vitamin A and the antirachitic factor, which was named vitamin D in 1925 as it was the fourth one to have been discovered.

McCollum’s demonstration that vitamin D deficiency was the cause of rickets did not settle one outstanding matter. In 1919, a Berlin physician, Kurt Huldschinsky, had cured rickets in children by exposing them to ultraviolet light emitted from a mercury vapour lamp. His results were corroborated the following year in Vienna by Chick’s group of lady doctors and scientists who, at the end of the war, had been sent from the Lister Institute to assist during a severe epidemic of rickets that affected four out of every five infants in the city. They found the disease did not develop in children exposed to adequate sunlight. Further confirmation came from New York, where Hess at the College of Physicians and Surgeons at Columbia University cured rachitic infants by exposing them to sunlight or ultraviolet radiation. Hess suggested that the antirachitic principle might be formed by the action of ultraviolet light on a putative provitamin. He went on to make the surprising discovery, announced in June 1924, that irradiation of certain foods could confer antirachitic properties on them. Before his report appeared in print in October of that year, Harry Steenbock of the University of Wisconsin published similar findings. He took out patents to cover the processing of foods by ultraviolet light, assigning these to a body established in 1925 to enable the vast sums
earned from license fees to be used in support of research in Wisconsin. This was the Wisconsin Alumni Research Foundation, which earned more than $14 million from Steenbock's patents during the next 20 years.

Hess and workers in several other laboratories soon established that the substance converted into vitamin D when vegetable oils were irradiated was to be found among the plant sterol fraction. He then went to Göttingen to work on the isolation of provitamin D under the guidance of Adolf Windaus. In 1927, Hess and Windaus, with the assistance of the Göttingen physicist Robert Pohl, established that the provitamin D was a known substance, namely ergosterol. The following year, Windaus was awarded the Nobel Prize for Chemistry in recognition of this and his earlier work on sterols. In collaboration with the Elberfeld laboratories of I.G. Farbenindustrie, in 1932 Windaus isolated the product formed by irradiation of ergosterol. He named it vitamin D$_2$, to distinguish it from what he had previously thought was the pure vitamin, namely its complex with lumisterol (a precursor also formed by irradiation of ergosterol). Windaus renamed that complex, calling it vitamin D$_1$ and then elucidated the chemical structure of vitamin D$_2$ in 1936.

A vitamin D$_2$ complex was also isolated in 1932 by Askew and his colleagues at the National Institute for Medical Research, London. At that time, this was believed to be homogeneous, and was mistakenly assumed to be identical to Windaus' vitamin D$_2$. It was given the name 'ergocalciferol'. The term 'vitamin D' is now used as a generic term to describe any substance that can be converted in the body into the active antirachitic metabolite 1,25-dihydroxycholecalciferol.

Vitamin D$_3$ (Cholecalciferol, Calciol)

Irradiation of other sterols was found to generate antirachitic products such as vitamin D$_3$, or cholecalciferol, which was formed from 7-dehydrocholesterol. Windaus found this sterol present in skin, thereby solving the mystery of how exposure to sunlight could prevent or cure rickets. Vitamin D$_3$ is the product formed on irradiation of foods from animal sources. It was synthesised in 1966 by Hector DeLuca and his colleagues at the University of Wisconsin-Madison.
DeLuca identified calcifediol as the major circulating metabolite of cholecalciferol. Since it was more potent than cholecalciferol and had a faster onset of action, it was introduced in the early 1970s for treating hypocalcaemia in hypoparathyroid patients and in those on renal dialysis. DeLuca also isolated calcitriol, another active metabolite of cholecalciferol. As it is more polar than other vitamin D analogues, its duration of action is shorter.

**α-Tocopherol (Vitamin E)**

In 1922, Herbert Evans and Katharine Bishop of the University of California, San Francisco, announced that normal pregnancies did not occur in rats kept for long periods on an artificial diet supplemented with all known vitamins. Few offspring were produced as most foetuses were resorbed a few days after conception. Evans suggested that this arose from deficiency of a substance that was eventually to become known as vitamin E. Much interest was aroused six years later when Evans and George Burr discovered that paralysis occurred in young rats whose mothers had been maintained on low levels of the vitamin during pregnancy. Wheat germ oil, a rich source of the vitamin, could cure the paralysis if administered to the rats shortly after their birth. Other workers later suggested this paralysis was a form of muscular dystrophy, leading to much controversy over the possible role of the vitamin in that disease. Matters were complicated by the instability of the vitamin preparations, which were sensitive to oxidation.
The pure vitamin was isolated by Evans and his colleagues in 1936 from a wheat germ oil concentrate.\textsuperscript{107} It was given the name $\alpha$-tocopherol (Greek: \textit{tokos} = childbirth, \textit{pherein} = to bear). The chemical structure was established two years later by Fernholz of Merck and Company\textsuperscript{108} and the vitamin was synthesised shortly after by Paul Karrer.\textsuperscript{109} Availability of the pure vitamin from natural or synthetic sources enabled researchers to establish whether it had any role in human nutrition or therapeutics. None of the many claims made for its therapeutic value in human diseases has ever been substantiated. The main value of $\alpha$-tocopherol appears to be its safety as an antioxidant for use by the pharmaceutical and food processing industries.

**Phytomenadione (Vitamin K\textsubscript{1})**

Henrik Dam carried out a series of experiments at the University of Copenhagen in 1929 to establish whether chickens could synthesise cholesterol, doubts previously having existed about this.\textsuperscript{110} He was able to confirm that cholesterol was indeed synthesised, but in the course of proving it he found that his chickens began to haemorrhage after two or three weeks on a fat-free diet supplemented with the known fat-soluble vitamins. Samples of their blood showed delayed coagulation. Dam doubted that this could be a form of scurvy since chickens were already known not to require vitamin C. Nevertheless, he added lemon juice to their diet, but to no avail. Only large amounts of cereals and seeds in the diet afforded protection. In 1934, he reported the existence of a new accessory food factor and then went on to show, in the following year, that this was fat-soluble, but different from vitamins A, D or E. He described it as vitamin K since it was required for blood coagulation.\textsuperscript{111} A substance with similar activity was discovered shortly after by H.J. Almquist and Robert Stockstad at Berkeley. They had discovered that alfalfa meal contained a factor that protected chickens against a scurvy-like haemorrhagic disease induced by being fed on diets in which the source of protein was sardine meal. Almquist was able to demonstrate that meat meal was satisfactory because its slower processing allowed bacterial production of an antihaemorrhagic factor. After this had finally been isolated, a report submitted to \textit{Science} was rejected.\textsuperscript{35} It was belatedly sent to \textit{Nature} where it appeared a few weeks after Dam’s paper had been published.\textsuperscript{112}

Dam sought the assistance of Paul Karrer at the University of Zurich in purifying vitamin K. They isolated it in 1939 as an impure oil.\textsuperscript{113} At the same time, a team led by Edward Doisy at the St Louis University School of Medicine separated two forms of the vitamin from vegetable and animal sources, naming them vitamin K\textsubscript{1} and vitamin K\textsubscript{2} respectively. They obtained pure vitamin K\textsubscript{1} as crystals from alfalfa and promptly determined its structure\textsuperscript{114} and then synthesised it.\textsuperscript{115} The synthesis was also accomplished by Louis Fieser at Harvard\textsuperscript{116} and by Almquist.\textsuperscript{117} Vitamin K\textsubscript{1} later received the approved name of ‘phytomenadione’, but it is also known as ‘phylloquinone’. Henrik Dam and Edward Doisy were awarded the Nobel Prize for Physiology and Medicine in 1943 for the discovery of vitamin K.
Vitamin K₂ was extracted from fishmeal by Doisy and his colleagues.¹¹⁸ It consisted of closely related compounds known as ‘menaquinones’, which are synthesised in the intestines by bacteria. They have up to 15 isoprene units in their side chain. The structure of menaquinone 4, which has four isoprene units, is shown.

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Following the introduction of the sulfonamides and antihistamines in the late 1930s and the 1940s, the idea that drugs may compete with natural metabolites at receptors gained wide recognition. The ‘lock and key’ analogy, popularised by Paul Ehrlich at the beginning of the twentieth century to explain how chemotherapeutic drugs interacted with their receptors, now came back into vogue. An essential feature of that hypothesis was the necessity for a potential drug to have a structural similarity to the substrate, or metabolite, that normally attached to the receptor in question. The drug could then block access to the receptor by the natural substrate and so act as an antimetabolite.

Only one vitamin has provided analogues that are used therapeutically as antimetabolites, namely folic acid. When antifolates were found to induce remissions in children with leukaemia, it encouraged chemists to synthesise a diverse range of antimetabolites that interfered with cell division. This remains a significant driving force behind the design of novel chemotherapeutic drugs.

FOLIC ACID ANALOGUES

Richard Lewisohn, a surgeon at the Mount Sinai Hospital in New York, initiated a series of experiments in 1937, hoping to establish why primary tumours of the spleen were rarely encountered. He began by injecting a concentrated beef spleen extract into mice bearing transplanted tumours (sarcoma-180) and observed complete regression of these in 60% of the animals. The extract had to be given subcutaneously as it was highly irritant and caused thrombosis if injected into a vein. It had no effect on spontaneous (i.e. naturally occurring) tumours in mice. Noting that the spleens of the healed mice were often enlarged, Lewisohn prepared extracts of these as well. Since the mouse spleen extract was dilute and non-irritant, it could be safely injected intravenously. Unlike the original beef extract, this induced complete regression of spontaneous breast tumours in 30% of mice. Although tumours reappeared in a quarter of the mice, never before had a non-toxic substance produced such a result.

Although encouraged by his findings, Lewisohn recognised that quantity production of the ‘healed mouse extract’ was impracticable. He began an extensive screening programme in 1939 to find other agents that could cause regression of tumours. An obvious step was to examine the recently discovered vitamins of the B group that were present in liver. Using yeast extract as a cheap, convenient source of these, he and his colleagues obtained positive results. Intensive investigations began in January 1941, requiring the use over the next three or four years of some 12 000 mice bearing sarcoma-180, and half that number bearing spontaneous tumours. When the yeast extract was injected intravenously once daily for up to ten weeks, approximately one-third of spontaneous breast adenocarcinomas regressed completely. Measurable changes were also detected when autopsies were conducted on mice that received four intravenous injections over a 48 hour period beginning a week or so after transplantation with sarcoma-180. With the entry of the United States into the war, it became impossible for Lewisohn to continue to obtain from Germany the brewer’s yeast used in the preparation of...
his extracts. Seeking alternative sources of active material, he found that barley extract was as efficacy as yeast extract. By this time such was the nature of his findings that the International Cancer Research Foundation, which had sponsored Lewisohn’s investigations, asked Cornelius Rhoads, Director of the Memorial Hospital in New York, to conduct an independent enquiry.3 This was duly entrusted to one of the senior scientists at the hospital, in 1943. His attempts to confirm Lewisohn’s findings were unsuccessful, no significant difference being detected either in the growth of sarcoma-180 in treated and untreated mice, or in the regression of spontaneous breast cancer.

Early in 1944, it occurred to Lewisohn that the active substance in yeast and barley extracts might be the newly isolated folic acid. His subsequent finding of antitumour activity in a folic acid concentrate did not settle the matter, since it contained many impurities. However, Lewisohn was able to obtain the support of Lederle Laboratories, who supplied small amounts of the scarce crystalline fermentation Lactobacillus casei factor they had just isolated. In an initial test on seven mice, each receiving 0.25μg by intravenous injection, Lewisohn observed the greatest inhibition of tumour growth he had ever seen. Extensive studies on mice with spontaneous breast cancer confirmed the initial results, these being published in January 1945.4 Subsequently, Lederle researchers discovered that the material supplied to Lewisohn was not identical to the liver L. casei factor. It was pteroylglutamic acid, whereas the liver factor was pteroylglutamic acid (now always described as folic acid).5 When the Mount Sinai Hospital researchers tested the liver L. casei factor (i.e. folic acid), they found it to be ineffective against spontaneous breast cancer in mice.6 This persuaded Lederle to synthesise both the di- and triglutamates for clinical trials. These were appropriately named Diopterin® and Teropterin®, respectively, and were sent to Farber for clinical evaluation. He and colleagues in three hospitals associated with the Harvard Medical School began by administering the drugs to 90 patients with a variety of malignancies that offered no hope of cure. The purpose of this phase I trial was to determine the toxicology, appropriate dosage and suitable routes of administration of the folic acid conjugates in humans.

After cautious initial studies, Teropterin® was given daily in doses up to 150mg intramuscularly, or 500mg intravenously, the average length of treatment being 35 days. Neither local nor systemic adverse effects were encountered. Although reluctant to draw conclusions about the efficacy of the treatment after so short a period of observation, Farber and his colleagues,7 in a brief preliminary report published in December 1947, suggested that the temporary improvements observed in some patients warranted further investigations.

During that phase I trial, biopsies of bone marrow had been routinely taken from several patients with acute leukaemia. Unexpectedly, these showed that the folates had accelerated the progress of the disease towards its fatal termination. On reviewing the situation, Farber
speculated whether this ‘acceleration phenomenon’ could be put to advantage in either of two
distinct ways.\(^8\) The first would be to use the folic acid conjugates to stimulate leukaemic cells
to grow and divide, thereby rendering them more susceptible to nitrogen mustard
chemotherapy. The second possibility would be to administer one of the antagonists of folic
acid that had been newly developed by Yellepragada Subba Row and his colleagues at Lederle
Laboratories.

A variety of antagonists of vitamins, hormones and cell metabolites had been synthesised
after Donald Woods of Oxford University discovered in 1940 that sulfonamides exerted their
antibacterial action by antagonising the role of 4-aminobenzoic acid, a growth factor for
bacteria.\(^9\) Such antagonists were described as antimetabolites. This phenomenon has first been
reported in 1931 by Judah Quastel at Cambridge when he introduced the term ‘competitive
inhibition’ to describe how malonic acid inhibited the oxidation of succinic acid by bacteria,
and also in brain and muscle tissue.\(^10\)

In 1947, Lederle researchers reported the synthesis and biological properties of a crude
methylated derivative of folic acid (‘\(\alpha\)-methyl pteroylglutamic acid’, a mixture of methylated
products) that was a weak antimetabolite.\(^11\) It had a depressant action on the blood-forming
elements of the bone marrow in rats and mice, as was anticipated from prior knowledge of the
effects of folic acid deficiency. The extent of this, however, was greater than previously seen,
the animals becoming not only anaemic but also exhibiting a reduction in the number of white
blood cells. When tested on a patient with chronic leukaemia, the antimetabolite was not
potent enough to produce any worthwhile result. The same chemists who had synthesised folic
acid at Lederle Laboratories now prepared more antimetabolites, the first of which,
pteroylaspartic acid, was sent to Farber.\(^12\)

At the Children’s Medical Center in Boston on 28 March 1947, Farber administered
pteroylaspartic acid to a four year old girl dying of acute myeloid leukaemia. She received
40 mg daily intramuscular injections until her death a week later. Post-mortem examination
revealed that although the treatment had not been started in time to save her life, the number
of leukaemic cells in her bone marrow had been drastically reduced. Commenting on this, in
their report in the *New England Journal of Medicine* a year later, Farber and his colleagues
stated, ‘A change of this magnitude in such a short time has not been encountered in the marrow of leukaemic children in our experience.’ The deaths throughout the world, despite treatment with the best available therapy, of that four year old girl and thousands after her were inevitable until the lessons painfully learned from them finally enabled Farber’s successors to conquer the most dreaded of all diseases of childhood.

Following the discovery that replacing the oxygen substituent on the 4-position of the pteridine ring by an amino group enhanced the potency of folic acid antagonists, aminopterin was synthesised at the Bound Brook laboratories of the American Cyanamid Company in New Jersey (Lederle Laboratories was the other main division). Farber had treated a further 14 children with pteroylaspartic acid before receiving aminopterin in November 1947. It was the first potent folic acid antagonist he had worked with. During the next six months, 16 children with acute leukaemia were treated with it. Many of them had been moribund at the onset of therapy, yet complete remissions were obtained in ten cases. These were the first sustained remissions ever obtained in leukaemic patients. The white cell counts had apparently returned to normal levels, with either a marked reduction or complete disappearance of the malignant blast cells (lymphoblasts). The red cell count had also approached normal values. Toxic effects were certainly present, the most frequent being a troublesome stomatitis affecting the rapidly dividing lining cells of the mouth, leading to painful ulceration. Notwithstanding this, aminopterin quickly became established as a major advance in the treatment of acute leukaemia.

In the summer of 1947, the Bound Brook researchers developed methotrexate. Once there was evidence that it was safer than aminopterin, it rendered all other antifolates obsolete. It also proved effective in treating choriocarcinoma, lymphomas and some solid tumours.

Two schools of thought now developed over the management of the leukaemic children in whom remission had been achieved. Some haematologists stopped administration of aminopterin as soon as the blood and bone marrow appeared normal, restarting treatment only when leukaemic cells reappeared, as they inevitably did. This process was repeated until no further improvement could be achieved. Farber had spurned this type of intermittent therapy, and opted for maintenance therapy with repetitive administration of aminopterin at regular intervals until resistance to the drug occurred. In this way, he kept his patients alive for an average of eight or nine months, and one in every hundred or so appeared to be cured. In the succeeding years, it became evident that Farber’s approach had been correct since remission did not represent elimination of the disease, but was merely a reduction in the number of leukaemic cells in the body to a level at which they were no longer detectable. It has been estimated that something in the order of 1000 million leukaemic cells were present at this stage; without further treatment these repeatedly divided until symptoms were experienced when the number had increased a thousandfold. Only by further exposure to regular cycles of therapy could the number of these remaining cells be kept under control. The problem facing Farber was to establish the maximum dosage of methotrexate that could be administered for several days without destruction of too many normal blood-forming elements in the bone marrow. When the white cell count fell below the critical level, the patient was exposed to the risk of death from overwhelming infection. To strike the right balance required constant monitoring of the bone marrow and blood.

Following the development of a variety of other antileukaemic drugs, including cortisone or prednisolone, an aggressive approach aimed at an outright cure of leukaemia in children was pioneered by Donald Pinkel, one of Farber’s former associates. Appointed medical director of the newly opened St Jude’s Children’s Research Hospital in Memphis, Tennessee, in 1962, he administered different combinations of several cytotoxic drugs to randomly allocated groups of patients who had received no prior treatment. The progress of these groups revealed the optimum treatment schedules. Among the advances made by Pinkel was his recognition that the frequent deaths from meningeal leukaemia among long-term survivors who had
received chemotherapy was due to the persistence of small numbers of leukaemic cells present in the brain at the onset of the disease. These had not been destroyed because of the poor penetration of the central nervous system by the cytotoxic drugs. Simply by irradiating the craniums of children after they first entered remission, he virtually eliminated this complication. By the early 1970s, more than half his patients were still alive five years after diagnosis of the disease. Today, the majority of children with acute lymphocytic leukaemia reach this stage, and most of them are cured. Prior to the introduction of the antifolate drugs, no child ever survived beyond three months after diagnosis.

In the 1970s, there was growing recognition of the success of combination chemotherapy in treating leukaemia. It strongly influenced the approaches being taken towards cancer chemotherapy. At the same time, medicinal chemists acquired an appreciation of the potential of antimetabolites as chemotherapeutic agents. By the end of the twentieth century there were many more of them in the pharmacopoeia.

**MODIFIED PURINES**

Soon after the introduction of sulfanilamide into clinical practice it was discovered that its antibacterial activity was antagonised by pus, as well as by tissue or yeast extracts. In 1940, Donald Woods at Oxford University had the idea that the substance responsible for this antagonism might be structurally similar to sulfanilamide, thereby acting in much the same manner as physostigmine and tubocurarine did in antagonising the action of acetylcholine, i.e. by competing with it for an unidentified receptor site. Woods showed that 4-aminobenzoic acid was a very effective antagonist of sulfanilamide, which led him to propose that sulfanilamide acted as an antimetabolite of 4-aminobenzoic acid. He emphasised that the antagonism depended on the close structural relationship between the two compounds.

George Hitchings of the Wellcome Research Laboratories in Tuckahoe, New York, appreciated the implications of the antimetabolite hypothesis. He had gained his PhD from Harvard in 1933 for work in the then-unfashionable field of nucleic acid metabolism. He joined Wellcome in 1942 and started to prepare and test potential antimetabolites of the pyrimidines required for the synthesis of nucleic acids. This was long before Watson and Crick had elucidated the central role of DNA in cellular reproduction. Hitchings' motivation had simply been his recognition that as all cells required nucleic acids to divide, it might be possible to block reproduction of rapidly dividing bacteria, protozoa or tumours by interfering with their synthesis of nucleic acids.

Hitchings and his colleagues initially examined more than 100 analogues of thymine for their ability to inhibit the growth of *L. casei*, an organism used in screening for drugs with antifolate or antipyrimidine activity. Although 5-bromouracil was found to have potent activity, it was never introduced into the clinic. The first success for the Wellcome researchers was not with an analogue derived from a pyrimidine, but with one from a purine.

**Mercaptopurine**

The work at Tuckahoe on nucleic acid antimetabolites continued with the examination of analogues of adenine, one of the two purines in DNA. Diaminopurine was synthesized in 1948 by Gertrude Elion and found to inhibit the growth of *L. casei*. Following encouraging animal tests, it was evaluated at the Sloane–Kettering Institute, then the leading centre for screening of potential anticancer agents against transplanted tumours. Subsequently, it underwent a full clinical trial at the Memorial Hospital in New York. Although an occasional remission was seen in children with acute leukaemia, diaminopurine was clearly inferior to aminopterin and methotrexate.
Elion synthesised mercaptopurine in 1950 as a chemical intermediate for the subsequent preparation of more aminopurines. When routine screening unexpectedly revealed it to possess outstanding activity as an inhibitor of the growth of *L. casei*, it was sent to the Sloane–Kettering Institute for further evaluation. This led on to a clinical trial at the Memorial Hospital, where it was established that mercaptopurine was the safest and most effective antileukaemic agent yet to have been discovered. Used on its own, the average remission lasted about one year. Of particular significance was the fact that a remission was even induced in one patient who had relapsed after failing to respond to further treatment with a folic acid antagonist. This indicated that there was no cross-resistance between the two classes of compounds and so provided the basis for the introduction of the combination chemotherapy that was to have such dramatic results in children with acute leukaemia.

By 1956, it was evident that a combination of cortisone with either methotrexate or mercaptopurine produced more remissions than any single drug, while also extending the duration of the remissions. By the end of the decade, the mean survival rate for children with acute leukaemia who had received intensive chemotherapy in specialised centres had risen beyond one and a half years. Advances since then have raised the cure rate to between 70 and 80% for those children with the commonest type of acute leukaemia who receive properly supervised combination chemotherapy.

**Azathioprine**

Much of the mercaptopurine administered to patients was rapidly metabolised before it could exert any therapeutic effect. This occurred through the action of the enzyme xanthine oxidase. Analogues containing substituents on the sulfur atom were synthesised by Elion and Hitchings in the hope of obtaining a prodrug that would provide a sustained release of mercaptopurine. The most active of these was azathioprine, synthesised in 1957. However, it gave disappointing results in a clinical trial. Nevertheless, it later became an important drug in its own right after the discovery of a new application for mercaptopurine.

Peter Medawar at University College London stimulated interest in immunological tolerance with his Nobel Prize winning work in the 1950s. At Tufts University School of Medicine in Boston, William Dameshek then had the idea that if a drug could be found that would be more effective than cortisone in depressing the immune response, it might be possible to carry out bone marrow transplantation in patients with aplastic anaemia, leukaemia or radiation damage. Assisted by Robert Schwartz, he examined a variety of drugs to assess their effect on the ability of rabbits to produce antibodies against injected human serum albumin. Mercaptopurine turned out to be highly effective, but when Roy Calne at Harvard Medical School tried it in dogs receiving kidney transplants, it was unable to induce the same
degree of immunological tolerance. Fortunately, Calne noted that the transplanted kidneys functioned for much longer than usual, no other drug having a comparable effect. In the light of this development, Hitchings set up a screening programme in which a variety of drugs was tested for their capacity to inhibit the haemagglutinin reaction in mice challenged with foreign red blood cells. From this screen, azathioprine emerged, in 1961, as the most effective drug. Calne went on to pioneer its use in human transplant surgery, and it became the most commonly used cytotoxic immunosuppressant until the introduction of ciclosporin.

**Allopurinol**

Having failed to find any analogues of mercaptopurine that were superior in the treatment of leukaemia, Elion and Hitchings turned to trying to enhance the activity of mercaptopurine by finding a compound that would inhibit xanthine oxidase, the enzyme responsible for its rapid destruction after administration. Allopurinol, in which one of the purine nitrogens had been moved to form a pyrazolopyrimidine, emerged from *in vitro* testing and was shown to diminish the conversion of mercaptopurine to inactive thiouric acid. Furthermore, it was devoid of cytotoxicity.

A clinical trial organised by Wayne Rundles of Duke University School of Medicine in North Carolina then found that, despite an enhancement of the antitumour activity of mercaptopurine in the presence of allopurinol, no therapeutic advantage was detected. However, Rundles recognised the potential of a xanthine oxidase inhibitor in interfering with the biosynthesis of uric acid. He tested it in the treatment of gout, where it prevented the formation of uric acid crystals in joints and thus brought relief to the victims of this painful condition. Allopurinol was then marketed for this purpose in 1966.

**Thiabendazole**

One of the leading exponents of antimetabolite therapy was Wayne Woolley, who worked at the Rockefeller Institute in New York. In 1944, he noted the resemblance of benzimidazole to adenine and speculated that it might act as an adenine antimetabolite. He even demonstrated that it could inhibit the growth of bacteria and fungi and that this could be reversed by either adenine or guanine. After this, several papers were published describing the *in vitro* activity of benzimidazole, but in 1953 researchers at the University of Michigan reported that subcutaneous injections of benzimidazole reduced mortality in mice experimentally infected with poliomyelitis virus. This encouraged researchers at Merck to synthesise benzimidazoles as potential antiviral drugs. Routine screening of these using a variety of assays for chemotherapeutic activity revealed that 2-phenylbenzimidazole had anthelminitic activity. Hundreds of analogues were then examined, from which thiabendazole emerged as one of the most potent chemotherapeutic agents ever discovered. A concentration as low as one part in a million was capable of preventing the development of *Ascaris* eggs *in vitro*. It became the drug of choice for the treatment of strongyloidiasis, a gut infection caused by the roundworm *Strongyloides stercoralis*, which occurs widely in tropical regions. Unless effective eradication is instituted, the larvae penetrate the gut wall to invade the tissues and a cycle of autoinfection ensues.
Merck researchers went on to seek an analogue of thiabendazole that would be more resistant to metabolic inactivation, hoping that this would permit higher plasma levels and thus better tissue penetration. This was to be achieved by preventing enzymatic hydroxylation at the 5-position of the ring system. However, straightforward substitution with alkyl, aryl and halo substituents was to no avail. When the 5-amino analogue of thiabendazole was found to lack activity, it was presumed that this was due to metabolic conversion of the amino group. To avoid this, carbamates were synthesised, resulting in the preparation of cambendazole. It proved to be a potent, broad-spectrum anthelmintic, which is valuable for the control of gastrointestinal nematode infection in animals.

A similar approach enabled Janssen researchers to introduce mebendazole, a broad-spectrum anthelmintic drug of which a single oral dose can eliminate threadworm, roundworm, whipworm or hookworm infections.

**MODIFIED PYRIMIDINES**

When Abraham Cantarow and Karl Paschkis at the Jefferson Medical College in Philadelphia reported in 1954 that radioactive uracil was more rapidly absorbed into experimental rat liver tumours than into normal liver cells, it caught the attention of Charles Heidelberger at the McArdle Laboratory for Cancer Research in the University of Wisconsin, Madison. He had previously shown that the fluorine atom in the metabolic poison fluorooacetic acid was responsible for inhibition of a vital enzyme, so he now decided to test the effects of incorporating a fluorine atom into uracil. He asked Robert Duschinsky and Robert Schnitzer of Hoffmann–LaRoche in Nutley, New Jersey, to synthesise fluorouracil and other similar pyrimidines. In 1957, they reported that fluorouracil had potent activity against transplanted tumours in rats and mice. Subsequent clinical studies have shown that its principal therapeutic role is in the treatment of gastrointestinal tumours and in combination chemotherapy for breast cancer.

Heidelberger was able to demonstrate that fluorouracil was metabolised to 5-fluorodeoxyuridine monophosphate (FdUMP). This then inhibited thymidylate synthetase, the enzyme that otherwise methylates the uracil in deoxyuridine monophosphate to form the thymine that
is incorporated into DNA. This led Heidelberger to test FdUMP, which was subsequently given the approved name of ‘floxuridine’. Although active against some tumours and viruses, it has been much less successful than fluorouracil in the clinic.

A major problem with fluorouracil was its variable oral bioavailability, leading to unpredictable results in the clinic. This is due mainly to it being metabolised in the gut wall and in the liver after absorption from the gut. The enzyme responsible for this is dihydopyrimidine dehydrogenase. Several prodrugs have been introduced that avoid metabolism by this particular enzyme, the first being tegafur (ftorafur). This was synthesised at the Taiho Pharmaceutical Company, Tokuahima, in Japan. It is converted to fluorouracil in the liver after absorption from the gut, without involvement of dihydopyrimidine dehydrogenase. Tegafur is used in colorectal cancer.

Doxifluridine was developed by Hoffmann–La Roche at Nutley. It releases fluorouracil in the presence of uridine phosphorylase, of which relatively high amounts were found in sarcoma-180 and thus accounted for the high activity against this solid tumour. Capecitabine is a prodrug designed by Hoffmann–La Roche chemists to release doxifluridine only after it had been absorbed from the gut. This reduces the risk of intestinal damage caused when fluorouracil or doxifluridine are given by mouth. The principal use of capecitabine at present is in metastatic colorectal cancer.

Flucytosine, which was also prepared by Hoffmann–La Roche chemists, is selectively converted to fluorouracil in fungi by cytosine deaminase, an enzyme absent from human cells. This has made it a valuable agent in the treatment of systemic yeast infections such as candidiasis, cryptococcosis and torulopsosis. These can be particularly hazardous in cancer patients whose immune system has been compromised as a consequence of exposure to intensive chemotherapy.

William Prusoff of the Department of Pharmacology at Yale prepared idoxuridine. Although this was too toxic for systemic use, it could be topically applied to treat skin infections caused by herpes simplex. In infected cells, viral thymidine kinase converts idoxuridine into its monophosphate, which then interferes with cell division. As mammalian thymidine kinase is much more selective, it does not accept idoxuridine as a substrate. However, the exact mechanism of action of idoxuridine is not yet fully understood.

Purine synthesis involves conversion of 5-aminoimidazole ribotide to its 4-carboxamide, which in turn is converted to the purine known as inosinic acid.
Dacarbazine was synthesised by Fulmer Shealy at the Southern Research Institute, Birmingham, Alabama. It was intended to be an antimetabolite by virtue of its structural similarity to the 5-aminoimidazole-4-carboxamide moiety in the precursor of inosinic acid. However, it was found that dacarbazine underwent metabolic $N$-demethylation in the liver and the resulting monomethyl compound spontaneously decomposed, forming 5-aminoimidazole-4-carboxamide and diazomethane. The latter is a well-known alkylating agent and in vivo it reacts with the 5-position of guanine in DNA. Since the alkylating activity is not limited to attack on guanine, dacarbazine proved to be highly toxic. Its use has been restricted to treatment of melanoma and in combination chemotherapy of Hodgkin’s disease and soft tissue sarcomas.

Dacarbazine is chemically unstable, especially in the presence of light. This necessitates it being administered by injection. To avoid this, temozolomide was developed by Malcolm Stevens and his colleagues in the Department of Pharmacy at the University of Aston, in association with Cancer Research UK and May & Baker Limited. It is currently given by mouth to patients with malignant glioma, the commonest and most lethal form of brain tumour, who have not benefited from other therapy. It remains to be seen whether other types of tumour will also respond to temozolomide.

Ribavirin is an analogue of the precursor of inosinic acid that was prepared by Roland Robins and his colleagues at ICN Pharmaceutical Corporation in Irvine, California. It was the first broad-spectrum antiviral drug, active against a variety of DNA and RNA viruses. It has been given by mouth in hepatitis C (in conjunction with interferon) and Lassa fever, as well as by inhalation in children with severe bronchiolitis caused by the respiratory syncytial virus.

MODIFIED NUCLEOSIDES

In 1951, Werner Bergman and Robert Feeney isolated spongothymidine, a novel type of thymine derivative, from a sponge (Tethya crypta) collected in the shallow waters off Elliot Key in Florida. Four years later, Bergman and David Burke isolated spongouridine and established the structure of both compounds, describing them as ‘spongou nucleosides’.

Because the spongou nucleosides resembled the nucleosides involved in DNA synthesis, there was interest in the possible anticancer activity of these and related synthetic compounds. The
only one that turned out to have useful clinical activity was cytarabine, which was synthesised in 1959 by Richard Walwick, Walden Roberts and Charles Dekker in the Biochemistry Department of the University of California at Berkeley. It was shown to possess activity against a transplanted sarcoma-180 by John Evans and his colleagues of the Upjohn Company. It has since found extensive use in the treatment of acute leukaemia.

In 1960, Bernard Randall Baker and his colleagues at the Stanford Research Institute in California synthesised adenine arabinoside, which became known as ‘vidarabine’. Although intended to be an anticancer drug, it found some value as an antiviral agent. By this time, it was apparent that the biological activity of drugs designed to interfere with DNA synthesis did not necessarily follow expectations. Screening against cancer cells, viruses, fungi, bacteria and even protozoal parasites was essential if potentially valuable compounds were not to be overlooked. That lesson had already been learned by Paul Ehrlich at the beginning of the twentieth century when his arsphenamine turned out to be an effective remedy for syphilis despite having been rejected as an antitypansomal agent.

Following the discovery of the activity of fluorouracil in 1957, John Montgomery and Kathleen Hewson at the Southern Research Institute synthesised 2-fluoroadenosine. It was so highly cytotoxic that it could not be used medicinally. Eleven years later, after the introduction of vidarabine, they prepared the arabinose analogue of 2-fluoroadenosine, which is now known as ‘fludarabine’. It is of value in B-cell chronic lymphocytic leukaemia. Cladribine is a deoxyadenosine analogue that was synthesised at Brigham Young University, Boston. It is given by intravenous infusion in hairy cell leukaemia or in some cases of chronic lymphocytic leukaemia.
In the early 1980s, Larry Hertel at the Eli Lilly laboratories in Indianapolis synthesised a number of deoxyribosides in which both hydrogen atoms in the 2'-position of the sugar rings were replaced by fluorine atoms. These were prepared as potential antiviral agents, but in 1990 it was reported that 2',2'-difluorodeoxycytidine was a very potent inhibitor of human leukaemia cells grown in culture. It is now known as gemcitabine and is administered intravenously in metastatic non-small lung cell carcinoma and pancreatic cancer.

Acyclic Nucleosides

In 1968, Burroughs Wellcome decided to extend their research to include antiviral agents after Frank Schabel at the Southern Research Institute in Birmingham, Alabama, had reported that adenine arabinoside was active against both DNA and RNA viruses. The arabinosides of 2,6-diaminopurine and of guanine were then prepared and found to be active against DNA and RNA viruses. As one of the company chemists, Howard Schaeffer, had previously demonstrated that the intact sugar ring of such nucleosides was not essential for binding to enzymes, acyclic analogues were synthesised and tested. In 1977, it was reported that one of them, aciclovir, had outstanding activity against the herpes virus.

Aciclovir is activated only in cells infected with herpes virus since these contain the key enzyme, viral thymidine kinase, that selectively converts the drug into a monophosphate. This, in turn, is converted by normal intranuclear kinases to aciclovir triphosphate. The triphosphate is incorporated into the DNA chain where it blocks further DNA chain extension as it lacks the key 2' and 3' carbon atoms needed for this to occur. The remarkable selective toxicity of aciclovir is due to the incapability of normal cellular thymidine kinase to accept it as a substrate.

Aciclovir is administered to treat superficial herpes simplex infections such as cold sores (by topical application) and genital herpes (by oral administration), or for treating life-threatening herpes varicella-zoster (chickenpox) infection in immunocompromised patients. Its safety having been demonstrated in over 30 million patients, aciclovir became available from United Kingdom pharmacies without prescription for the treatment of cold sores in 1993.

Ganciclovir was synthesised at the Syntex laboratories in 1980 as an analogue of aciclovir with a closer structural resemblance to natural nucleosides than had aciclovir. As a consequence, it turned out to be a better substrate for both viral and normal kinases involved.
in the formation of its triphosphate. This led both to a wider spectrum of antiviral activity and a greater degree of toxicity towards uninfected cells. This toxicity has limited its clinical application to life-threatening infections that do not respond to aciclovir or other agents. In particular, it has been administered in attempts to prevent blindness from peripheral retinitis in AIDS patients infected with the cytomegalovirus.

Penciclovir is an analogue of ganciclovir with similar activity to aciclovir against herpes simplex. It appears to have a safety profile not unlike that of aciclovir because of its lack of effect on DNA synthesis in uninfected cells. The closer structural similarity to natural nucleosides makes it a superior substrate for viral thymidine kinase, resulting in an enhancement of phosphorylation. Any advantage gained from this is counterbalanced by the reduced activity of penciclovir triphosphate as an inhibitor of DNA synthesis.

Famciclovir is a prodrug of penciclovir that was introduced in 1994. It was prepared at Beecham Research Laboratories in order to overcome the poor oral bioavailability of penciclovir. After absorption, it is metabolised in the liver, the ester functions being hydrolysed and the purine ring oxidised, to form penciclovir. It only needs to be administered three times a day by mouth, whereas aciclovir requires five daily doses.

Zidovudine

The clinical success of cytarabine, and to a lesser extent of vidarabine, drew attention to the potential of nucleoside analogues with a modified ribose ring. The importance of one such compound, azidothymidine, was to be overlooked for 20 years.

Azidothymidine was synthesised at the Michigan Cancer Foundation in 1964 by Jerome Horwitz as a potential antileukaemic drug. Its azido group was considered to be an appropriate substitute for the hydroxyl group in thymidine. After it gave negative results and was toxic when tested against L1210 leukaemia in mice, there seemed to be no point in further investigation. Ten years later, however, Wolfram Ostertag at the Max Planck Institute in Göttingen found azidothymidine shared the ability of another thymidine analogue, bromodeoxyuridine, to inhibit replication of the Friend leukaemia virus in cultured mouse cells. Even though this was a retrovirus, a type that transmits its genetic information through RNA rather than DNA, there was little interest in his report since retroviruses were then unknown in humans. The situation dramatically changed in 1983 when scientists at the Institut Pasteur in Paris isolated the retrovirus that is now known as the human immunodeficiency virus (HIV). By April of the following year, it had become apparent that HIV was responsible for the rapidly developing epidemic of the acquired immunodeficiency syndrome (AIDS). In those who had contracted AIDS, the HIV infected their T4 white blood cells that were crucial for development of an immune response to infections. The death rate among victims soared throughout the world.

The Burroughs Wellcome researchers were able to respond swiftly to the crisis because of their previous experience in screening for antiviral activity. In June 1984, virologist Marty St Clair set up a programme to identify drugs that had the potential to attack HIV by screening them against immortalised mouse cells infected with either the Friend leukaemia virus or the
Harvey sarcoma virus. These retroviruses were considered to be sufficiently comparable to HIV for the task in hand. The responsibility for selecting appropriate compounds for screening was given to nucleoside chemist Janet Rideout. Azidothymidine was one of the 14 compounds she chose. Her decision was to set in motion a sequence of events that successfully delivered a life-prolonging drug to AIDS patients 18 months later.

When the first laboratory results for azothymidine were obtained on 16 November 1984, they were so exceptional that St Clair thought she had omitted to add the Friend leukaemia virus to the cultures! When the results of testing against the Harvey sarcoma virus with a lower concentration of the drug were received two weeks later, they showed that there had been almost complete inhibition of virus replication. This aroused considerable interest and resulted in the speedy collation of all the previous findings when the company had examined azidothymidine as a potential antibacterial agent. These revealed that it had low toxicity when given to rats for two weeks. There was also evidence that it acted on DNA synthesis in bacteria but not in mammals. The decision was now taken to send samples of azidothymidine to the US National Cancer Institute (NCI) in Bethesda, Maryland, for further evaluation by Samuel Broder and Hiroaki Mitsuya. They were the first researchers to develop a method of testing compounds for activity against HIV, growing the virus in immortalised human T4 cells. Within two weeks of receiving azidothymidine from Burroughs Wellcome, they had concluded that it was highly effective.

The results from the NCI were confirmed at Duke University and the findings of the three laboratories involved were submitted for publication on 28 June 1985. The following month the Food and Drug Administration permitted a phase I clinical trial to proceed. The purpose was to establish safe dose levels with different routes of administration. The results indicated that the drug was safe enough for further investigation. In January 1986, a randomised, double-blind clinical trial on 282 patients began. It had been planned to last for 24 weeks, but after only 16 weeks the independent monitoring board stopped the trial since it had become apparent that among those receiving the drug only one of 145 had died, whereas 16 of those receiving placebo (dummy capsules) had died. As a result of these findings, supplies of azidothymidine were released in October 1986 for treatment on a named patient basis, with the Food and Drug Administration granting a product licence the following spring. Azidothymidine now received the approved name of ‘zidovudine’. Two major trials, the US Veterans Affairs Cooperative Study and the Anglo French Concorde Study, confirmed its ability to prolong the lives of those with AIDS or delay its development in those infected with HIV. Since these trials were completed, the practice has been to administer zidovudine as one of a cocktail of anti-HIV drugs. The outcome of this is to prolong the lives of those infected indefinitely, so long as the medication is continued.

As HIV is a retrovirus it contains RNA and must form viral DNA if it is to take over control and replicate inside the host cell. The enzyme that transcribes RNA into a DNA copy inside the host cell is known as reverse transcriptase. It is inhibited by zidovudine triphosphate which, once incorporated in the viral DNA chain, stops more nucleotides from being added since the azido group prevents the creation of the phosphodiester linkages required for the completion of the DNA chain. However, zidovudine triphosphate also affects mammalian DNA polymerase, for which it has one-hundredth the affinity of that for viral reverse transcriptase. This causes considerable toxicity, notably dose-dependent suppression of bone marrow, resulting in anaemia and leucopenia, which causes treatment to be abandoned in many patients.

**Dideoxynucleosides**

Jerome Horwitz and his colleagues in Chicago prepared the dideoxynucleoside now known as ‘zalcitabine’ three years after they synthesised zidovudine. This was also shown by Samuel
Broder and Hiroaki Mitsuya to prevent replication of HIV.\textsuperscript{71} The active form of zalcitabine is its triphosphate. In the nucleus this terminates DNA chain extension since there is no 3'-hydroxy group on its sugar ring to allow formation of phosphodiester linkages. It is now used in combination with other antiretroviral drugs in the treatment of AIDS, as is its sulfur analogue lamivudine.\textsuperscript{72}

Dideoxyadenosine, the corresponding adenosine analogue of zalcitabine, was synthesised by Roland Robbins in 1964.\textsuperscript{73} It was shown to have anti-HIV activity at the same time as zalcitabine, but was less potent and caused kidney damage. Didanosine was prepared from it by enzymatic oxidation and was also found to be active against HIV, but without causing kidney damage.\textsuperscript{74} It is also used in combination with other antiretroviral drugs for AIDS.

Glaxo Wellcome researchers at Stevenage in the United Kingdom and in North Carolina (to where Wellcome had moved from Tuckahoe in 1970) collaborated in the investigation of potential anti-HIV drugs in which the oxygen atom in the sugar ring had been replaced by a carbon in order to obtain compounds that were more stable \textit{in vivo} by virtue of not possessing a glycosidic linkage. Minimal activity was first observed in the carbocyclic analogues of dideoxyadenosine and four other nucleosides. Many nucleoside analogues were prepared and examined, but the only one that had significant activity and satisfied the requirements for use in the clinic was the 2',3'-didehydro analogue of dideoxyadenosine. Insertion of a cyclopropyl group on its 6-amino nitrogen of the adenine ring increased lipophilicity and thereby enhanced brain penetration. The resulting compound, known as abacavir, is used in combination with other antiretroviral drugs in the treatment of AIDS.

**HIV PROTEASE INHIBITORS**

Soon after the discovery of HIV, it was confirmed that it functioned like other retroviruses, so that once the double-stranded DNA is formed by the virus it is inserted into the host genome, a process facilitated by the action of viral integrase. The altered DNA then produces a polyprotein that is broken down to form smaller proteins which function as enzymes essential...
for HIV multiplication. Viral proteases that promoted this process were characterised in the mid-1980s.\textsuperscript{78}

In 1987, investigators at the Smith Kline and French Laboratories in Philadelphia prepared \textit{Escherichia coli} recombinant HIV protease and confirmed not only that the cleavage of the polyprotein in HIV was catalysed by HIV protease but also that among the enzymes formed were reverse transcriptase, viral integrase and HIV protease itself.\textsuperscript{79} In February 1989, Merck Sharp and Dohme Research Laboratories in Rahway, New Jersey, published the three-dimensional structure of HIV-1 protease.\textsuperscript{80} This major breakthrough was achieved so quickly because of the extensive work that had been taking place in commercial, academic and government laboratories around the world.\textsuperscript{81}

Roche Products in the United Kingdom was one of several groups that now sought non-toxic inhibitors of HIV protease. The approach they followed was similar in principle to that taken previously in the design of the ACE inhibitor captopril (see page 280), except that this time non-hydrolysable analogues of the dipeptides Phe–Pro and Tyr–Pro were sought since these occurred at the points of cleavage of the viral polyprotein that was formed in cells infected by HIV. This in itself was auspicious, since no mammalian protease was able to cleave such moieties; hence the expectation was that any effective inhibitor would be selective for the viral protease. Disappointing results were obtained with the first analogues, in which the amide linkage $R\text{-}CO\text{-}NH\text{-}R'$ (where $R$ and $R'$ are peptide chains) had simply been reduced to form $R\text{-}CHOH\text{-}NH\text{-}R'$. However, promising inhibitors were found when hydroxyethylamines with an interposed carbon atom, $R\text{-}CHOH\text{-}CH_2\text{-}NH\text{-}R'$, were prepared. The stereochemical configuration of the carbon atom bearing the hydroxyl substituent proved critical. Ultimately, the most active inhibitor, saquinavir, was obtained when the proline residue was replaced by decahydroisoquinoline-3-carbonyl.\textsuperscript{82} Saquinavir was the first HIV protease inhibitor to be marketed in the United States, in December 1995. An oral dose of 600 mg was prescribed three times a day in combination with a reverse transcriptase inhibitor and lamivudine. By this time, the importance of combining drugs with different modes of action in order to minimise the risk of viral resistance had been widely recognised.

\begin{itemize}
\item saquinavir
\item indinavir
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Merck’s indinavir incorporated a hydroxyethylene moiety (R–CHOH–CH₂CH₂–R’) and included a basic piperazine ring to enhance oral bioavailability. It was also administered thrice daily by mouth. Abbott Laboratories introduced ritonavir, the first protease inhibitor to be marketed in the United Kingdom. It was administered twice daily.

Clinical results have confirmed that treatment combining protease inhibitors with reverse transcriptase inhibitors dramatically reduces the HIV viral load to innocuous levels. Since an estimated 10¹² viruses are estimated to be formed daily before treatment, this is remarkable. As a consequence, AIDS can now be held at bay for many years – so long as chemotherapy is continued. The cost of treatment has been very high and in many countries it has been unaffordable and so patients continue to die of AIDS. Even when public pressure on companies persuaded them to waive their patent rights, lack of a robust local infrastructure for providing health care often negated the benefits of therapy.

**NEURAMINIDASE INHIBITORS**

The influenza virus consists of DNA coated with two proteins, neuraminidase and haemagglutinin. After the DNA replicates inside an infected cell, the haemagglutinin of the daughter viruses binds to glycolipid on the outside surface of the infected cell. Infection of other cells can only take place after neuraminidase has promoted the breakdown of the bonds holding the haemagglutinin to the sugar moiety of the glycolipid on the cell surface. The importance of inhibiting neuraminidase was recognised in the 1960s by Meindl and Tuppy, who prepared 2-deoxy-2,3-dehydro derivatives of sialic acid (also known as N-acetylneuraminic acid), the natural substrate for the enzyme. These were inactive in live animals.

Once neuraminidase had been crystallised by Graeme Laeve at the John Curtin School of Medical Research in the Australian National University, molecular models revealed a slot in the enzyme that was common to all strains of the virus. Attempts to develop inhibitors that would bind within this slot were initiated in 1986 by Mark van Itzstein at the Victorian College of Pharmacy in Melbourne and Peter Coleman of the ANC. Their approach was to use molecular modelling on a computer to establish how sialic acids bound to neuraminidase. This revealed that binding of the 2-deoxy-2,3-dehydro sialic acids made by Meindl and Tuppy could be enhanced by changing a hydroxyl group to an amino group. Further enhancement of activity was achieved by increasing base strength by replacing this amino group with a guanidine group to form zanamavir.
Zanamavir was evaluated at the laboratories of Biota in Melbourne and shown to be of value in the treatment of influenza. The development and commercialisation of it was subsequently licensed to Glaxo Wellcome, who marketed it in 1999. As a direct consequence of its origin from a carbohydrate, zanamir was too polar to be taken by mouth. Instead, it had to be administered from a dry powder inhaler. When inhaled twice daily for five days, it reduced influenza symptoms by 1–2.5 days.

Compound GS 4071 was developed as a neuraminidase inhibitor with enhanced lipophilicity. Even though the strongly basic guanidine group and the polar hydroxyls of zanamavir were absent it also had poor oral bioavailability. Unlike zanamavir, its ethyl ester was now sufficiently lipophilic to serve as an orally active prodrug. It received the name ‘oseltamavir’. The activity is similar to that of zanamavir, but it can be given by mouth to relieve the symptoms of influenza.

THE SAFETY OF DRUGS

The development of the HIV protease inhibitors generated considerable enthusiasm in some quarters, where it was naively believed that a host of inhibitors of proteases involved in other systems would soon become available. The failure of this to happen reflects the basic problem with all drug research – not merely that involving antimetabolites or substrate analogues – namely that while it is relatively easy to design active drugs, it remains exceedingly difficult to develop safe, effective medicinal compounds. No matter how much specificity for a single target a drug molecule may appear to have, there can never be a guarantee that it will not unexpectedly interact with a receptor site or enzyme somewhere else in the body.

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Towards the end of the nineteenth century, several European investigators found that the clotting of blood was accelerated by material extracted from body tissues with fat solvents. In 1911, however, Doyon in France discovered that aqueous extraction of de-fatted dog liver yielded an anticoagulant that he called antithrombine. He studied its effects over the next 15 or so years, but his findings were overlooked when William Howell, the Professor of Physiology at Johns Hopkins University in Baltimore, isolated a similar material in 1922. Howell called his product ‘heparin’, a name he had previously given to an inferior anticoagulant extracted from liver by fat rather than aqueous solvents. This earlier product had been discovered in Howell’s laboratory in 1916 after Jay McLean, a pre-medical student, had let his extracts of fat-soluble coagulating substance deteriorate and so unmask the presence of an anticoagulant. Howell spent some time trying to isolate and purify McLean’s phospholipid anticoagulant before switching to the use of aqueous solvents for liver extraction.

HEPARIN

During the 1920s, Howell studied the chemistry and physiological activity of his water-soluble heparin, characterising it as a sulfur-containing polysaccharide. He even persuaded the Baltimore firm Hynson, Westcott, and Dunning to sell it for laboratory investigations. This product is now known to have contained less than 1–2% of active material. Attempts were made to use it as an anticoagulant while transfusing blood into patients, but side effects were caused by impurities. Nevertheless, this brought it to the attention of researchers in Canada and Sweden.

In 1928, David Scott and Arthur Charles at the Connaught Laboratories in the University of Toronto joined in the effort to prepare samples of heparin that would be sufficiently pure for clinical investigations. By 1933, they had completed an investigation into the amounts of heparin in different animal tissues, which pointed to beef lung as the best source of the anticoagulant. However, the product extracted from this was different to that originally introduced by Howell. During the next three years, Scott and Charles worked with this new heparin and made a series of advances that finally provided material pure enough for clinical studies. This received recognition when an international standard for the sodium salt of heparin was published in 1935. Heparin prepared by Albert Fischer at the University of Copenhagen and by Erik Jorpes at the Caroline Institute in Stockholm also met the new standard.
Jorpes found heparin was an acidic sulfated polysaccharide that interfered with thrombin formation.\textsuperscript{9} The number of sugar units varied according to the source of the heparin, but was usually in the range of from 12 to 20.\textsuperscript{10}

Gordon Murray\textsuperscript{11} initiated clinical trials in Toronto with a view to establishing the value of the purified heparin in preventing thrombosis after injuries. Around the same time, Clarence Crafoord injected patients in Stockholm with it to prevent postoperative thrombosis.\textsuperscript{12} Charles Best went on to use heparin in exchange transfusion of blood between patients and donors in Toronto, just two years before the outbreak of the Second World War, when this technique was to save many lives. Purified heparin also facilitated the introduction of the artificial kidney in 1944, as well as the development of heart–lung bypass techniques in surgery.

The recognition that only part of the heparin molecule was required to inhibit blood clotting factor Xa led to the manufacture of low molecular weight heparin fractions by gel filtration.\textsuperscript{10} These retained the activity of heparin while overcoming its short duration of action. Later, enoxaparin was prepared by depolymerisation of porcine heparin.\textsuperscript{13} Apart from only requiring once daily injections, it is less likely to cause haemorrhage since it does not possess the longer-chain polysaccharide residues that inhibit other clotting factors.\textsuperscript{14}

**ALTEPLASE (TISSUE-TYPE PLASMINOGEN ACTIVATOR)**

Plasmin is present in blood to prevent unwanted clotting by catalysing the breakdown of the fibrin polymer that provides the framework of a blood clot. Plasmin is formed from plasminogen, a process that occurs after plasminogen has been activated by forming a complex with fibrin. After certain tissue fragments had been shown to possess plasminogen-activating ability, a soluble fraction possessing this activity was extracted and purified.\textsuperscript{15} Cloning and expression of complementary DNA (cDNA) for human tissue-type plasminogen activator permitted commercial production of alteplase.\textsuperscript{16}

Alteplase was shown to be a potent thrombolytic agent by virtue of its ability to activate plasminogen, thus breaking down fibrin in the thrombus.\textsuperscript{17} An early clinical study in patients with coronary occlusion following myocardial infarction showed that it was an effective drug for dissolving clots in coronary arteries.\textsuperscript{18} It was marketed for this purpose in 1988.

**APROTININ**

Aprotinin is a non-specific serine protease inhibitor originally found in bovine lung tissue and pancreas at the University of Munich in 1930.\textsuperscript{19} Six years later a trypsin inhibitor was also isolated from bovine pancreas.\textsuperscript{20} In 1959 Bayer patented aprotinin obtained from parotid glands where it occurred in greater concentrations.\textsuperscript{21} After purification, aprotinin was found to be a single-chain polypeptide containing 58 amino acids.\textsuperscript{22} In addition to its antifibrinolytic activity arising from its ability to inhibit plasmin, aprotinin inhibits various other proteolytic enzymes, including trypsin and kallikreins. It is licensed for treatment of life-threatening
haemorrhage arising from hyperplasminaemia and also for the prevention of blood loss during surgery.

EPOETIN

Following the introduction of thyroid extract and adrenaline, there was considerable interest in finding humoral factors that controlled other functions in the body. The first evidence of the presence of a factor that regulated red cell formation was obtained at the University of Paris in 1906 by Carnot and Deflandre, who reported the presence in the serum of recently bled animals of what they called ‘hemopoietine’, a substance that provoked marked hyperglobulism in normal animals. Other workers were unable to replicate the work of Carnot and Deflandre until 1953, when Erslev modified their method by infusing large volumes of plasma from extremely anaemic rabbits into normal rabbits. This study renewed interest in isolating what was now called ‘erythropoietin’. In 1960, erythropoietin was obtained from the plasma of anaemic sheep and a decade later from the urine of anaemic humans. Human erythropoietin, which differed slightly from that from sheep, was purified in 1977 by researchers at the University of Chicago using chromatographic and immunoadsorption techniques, and a partial amino acid sequence was established. From this development, cDNA probes were constructed in order to identify the gene; it was then cloned and expressed in bacteria (Escherichia coli). Once recombinant human erythropoietin became available, therapeutic application was possible. The recombinant product was given the approved name of ‘epoietin’.

The full structure of epoietin was determined in 1985 after it was shown to be an acidic glycoprotein featuring hexoses and hexosamines with sialic acid. The protein consisted of 166 amino acids, the sequence of which was deduced from the structure of the nucleotide sequence of cloned foetal hepatic erythropoietin cDNA. Two types are commercially available, alpha-epoietin and beta-epoietin. They are interchangeable for clinical purposes.

The first results from trials of epoietin to compensate for the deficiency of erythropoietin in patients with chronic renal failure as a result of damage to the sites of its formation in the kidneys were reported in 1986. Since then, the availability of epoietin has greatly reduced the need for blood transfusions in patients with chronic renal failure.

COLONY-STIMULATING FACTORS

Bone marrow cells divide rapidly to generate the billions of red and white cells that replace blood cells as they degenerate. However, bone marrow harvested from human donors could not be made to develop in vitro until the 1960s.

Following the discovery that colonies of malignant sarcomas, but not normal fibroblasts, grew on an agar medium, Ray Bradley at the Peter MacCallum Hospital in Melbourne tried to grow thymic lymphomas. This approach failed. He attempted to stimulate growth of colonies by inserting a variety of tissue fragments or cell suspensions in the agar layer. When a suspension of bone marrow cells was incorporated in the agar, colonies of cells at last grew. However, these were not found in the lymphoma cell layer as expected, but instead were developing within the bone marrow layer in the agar. This striking observation led Bradley and Don Metcalf to conduct a detailed examination which revealed that the new colonies consisted of macrophages whose growth was stimulated by substances diffusing from a variety of different tissues incorporated in the agar feeder layer. From these observations, it was realised that haemopoietic cell proliferation required stimulation by unknown factors and that a technique for investigating these was at hand. Metcalf and Bradley went on to show that blood serum contained growth factors that stimulated marrow cells to divide. Further investigation in several laboratories confirmed that serum contained a variety of these colony-
stimulating factors. Each was originally named after the specific type of cell whose growth it was found to stimulate, but subsequent work has shown that each colony factor can stimulate growth of more than one cell type.

The first stage of progress towards isolation of haemopoietic growth factors was to devise methods for culturing the specific types of cell lines involved. This was followed by the isolation of glycoproteins that proved to be the factors that stimulated growth. The breakthrough which finally opened the way for therapeutic application of these glycoproteins was the cloning of the genes regulating their production.33

The first of the stimulating factors to be marketed was granulocyte colony-stimulating factor. This was a relatively selective factor that mainly stimulated the proliferation and differentiation of bone marrow stem cells to form neutrophils. Metcalf and his associates purified it from mouse lung conditioned media and subsequently obtained the human factor.35 Human factor cDNA was cloned and then expressed in bacteria (E. coli), thus permitting commercial production.37 The recombinant granulocyte colony-stimulating factor was given the approved name of ‘filgrastim’. In contrast to the natural product, it has an amino-terminal methionine residue and was not O-glycosylated.

Filgrastim was developed by the American biotechnology company Amgen, working in collaboration with Hoffmann–La Roche. The first report of its use in the clinic appeared in 1987, when it was shown to reduce the number of episodes of infection during chemotherapy for advanced lung cancer. It was licensed in 1991 for shortening the duration of neutropenia following cancer chemotherapy or bone marrow transplantation.

Granulocyte macrophage colony-stimulating factor was purified in 1977 from murine lung conditioned media.38 Seven years later, highly purified material was isolated from a leukaemic human T-cell line in sufficient amounts to permit partial amino acid sequencing to be carried out.39 From this development, it became possible to construct cDNA probes to identify the gene from a DNA library. Human factor cDNA was then cloned and expressed in bacteria (E. coli), permitting commercial production.40 The recombinant granulocyte macrophage colony-stimulating factor, which was developed by scientists at Schering Plough’s Palo Alto Research Institute, received the approved name of ‘molgramostin’ and was marketed in 1992. It stimulates the production of monocytes, from which macrophages develop, and also that of granulocytes. Like filgrastim, it has been used in the clinic to shorten the duration of neutropenia following cancer chemotherapy or bone marrow transplantation.

**INTERFERONS**

The interferons are a family of proteins found by Alick Isaacs and Jean Lindenmann at the National Institute for Medical Research in London to confer resistance to a variety of viral infections.41 The discovery was made during an investigation into viral interference, being produced when inactivated influenza virus interacted with chick chorioallantoic membrane. Further investigation revealed that live viruses could stimulate cells to produce interferons that are species specific. These were found to exert complex effects on immunity and cell function. Three antigenically distinct interferons with different physical properties, respectively known as interferon alpha, beta and gamma, have been introduced into medicine as they have been found to exhibit antitumour activity.

When cultures of human leucocytes were exposed to a virus, interferons were produced. The major one, interferon alpha, was purified in 1974.42 An amino acid analysis was carried out in 1979, permitting cDNA to be cloned and expressed in bacteria. Commercial production was then feasible, subtypes also being produced.43,44 Two interferon alpha products have been evaluated in the treatment of viral infections and malignancies, including hairy cell leukaemia and Kaposi’s sarcoma in AIDS patients. They have received product licenses for such uses and several other purposes.
When cultures of human fibroblasts were exposed to a virus, interferon was produced. Interferon beta was isolated and then purified.\textsuperscript{45} Following the establishment of the amino acid sequence, cloning of cDNA and expression in bacteria led to the production in 1980 of recombinant interferon beta.\textsuperscript{46–48} It has been used in the treatment of colorectal cancer. Recombinant interferon gamma has been similarly obtained.\textsuperscript{49} It is given together with antibiotics to patients with chronic granulomatous disease.

**UROKINASE**

In 1861, the physiologist Ernst Wilhelm von Brucke in Vienna discovered that human urine had proteolytic activity.\textsuperscript{50} This was confirmed by Sahli, who noted the ability of urine to decompose fibrin.\textsuperscript{51} However, it was not until 1947 that the enzyme responsible for these observations was isolated from urine by Gwyn MacFarlane in Oxford.\textsuperscript{52} The name ‘urokinase’ was given to the enzyme and it was shown to activate plasminogen to form plasmin rather than simply to digest fibrin. A crystalline preparation was obtained in 1965.\textsuperscript{53} It is used to dissolve pulmonary embolisms.

**BILE ACIDS**

Chenodeoxycholic acid (chenodiol) was isolated in 1924 from goose gall by Adolf Windaus\textsuperscript{54} and human gall by Heinrich Wieland.\textsuperscript{55} Its complete structural configuration was elucidated by Hans Lettré at the University of Göttingen.\textsuperscript{56} In 1968, William Admirand and Donald Small at Boston University Medical School established that in patients with gallstones their bile was saturated with cholesterol, sometimes even exhibiting microcrystals, whereas this was not the case in normal people.\textsuperscript{57} It was then found that biliary levels of cholic acid and chenodeoxycholic acid were lower in patients with cholesterol gallstones than in normal people. Leslie Thistle and John Schoenfield at the Mayo Clinic in Rochester, Minnesota, then administered individual bile salts by mouth for four months and found that chenodeoxycholic acid reduced the amount of cholesterol in the bile.\textsuperscript{58} This led to a national collaborative study in the United States, which confirmed the effectiveness of chenodeoxycholic acid in bringing about dissolution of gallstones in selected patients. However, recent developments such as laparoscopic cholecystectomy and endoscopic biliary techniques have curtailed the role of chenodeoxycholic acid and ursodeoxycholic acid in the treatment of cholelithiasis.

Researchers in Japan investigated ursodeoxycholic acid (ursodiol) since it was a metabolite of chenodeoxycholic acid. Ursodeoxycholic acid had been isolated from bear bile in 1927 at Okayama University by Shoda, a product with a long history of use as a cholangogue in Japanese folk medicine.\textsuperscript{59} The structure was elucidated by Iwasaki.\textsuperscript{60} It proved to be four times as potent as chenodeoxycholic acid and of similar efficacy in selected patients with gallstones.\textsuperscript{61}
Hypocholesterolaemic Drugs

The French biochemist Joseph Redel synthesised 80 compounds that supposedly retained features of rings C and D of dehydrocholic acid. However, as these compounds contained an aromatic system, any resemblance to deoxycholic acid must be considered fanciful. Notwithstanding, the clinician Jean Cottet found that phenylethylacetic acid and several other disubstituted acetic acids lowered cholesterol levels in rats and humans. In 1953, Cottet and his colleagues published three papers on the clinical trials of these acids. Four years later, ICI researchers screened a variety of similar compounds that the company had previously prepared as plant hormone analogues and discovered high hypocholesterolaemic activity in clofibrate.

With increasing concern about the relationship between cholesterol levels and cardiac disease, clofibrate soon became the most frequently prescribed lipid-lowering drug. It was particularly recommended for use in the treatment of Type III hyperlipoproteinaemia. However, a major World Health Organization sponsored clinical trial found an association between chronic use of clofibrate and excess non-cardiovascular and total mortality. Consequently, it was replaced by safer analogues.
Gemfibrozil was introduced by Parke, Davis and Company.\(^6^4\) It was of value in the prevention of coronary heart disease in patients with very high cholesterol levels.\(^6^5\) Fenofibrate was more potent than gemfibrozil, but this is of little clinical significance.\(^6^6\) Bezafibrate had similar properties to fenofibrate.\(^6^7\)

**Antidiabetic Drugs**

In 1975, researchers at the Takeda Laboratories in Osaka synthesised 71 clofibrate analogues containing a biphenyl ether moiety as they were aware that both this and alkanoic acids were often present in experimental hypolipidaemic drugs. Several of the compounds they made had not only hypolipidaemic properties but also gave positive results when screened for hypoglycaemic activity. A chlorine atom at the alpha-position and an aryloxy or aralkyloxy substituent on the beta-aryl moiety enhanced hypoglycaemic activity in mice. Further testing in obese and diabetic mice revealed that AL-321 increased the insulin sensitivity of adipose tissue.\(^6^8\) Analogues of this were prepared, of which the most promising was a thiazolidine derivative, AL-294. Once again, more analogues were prepared, resulting in the development of ciglitazone.\(^6^9\) Although intended for clinical evaluation, it was finally considered to lack potency. Yet again, more analogues were prepared, finally resulting in the marketing of pioglitazone almost a quarter of a century after work had begun on clofibrate analogues.\(^7^0\)

![Chemical structures](image)

SmithKline Beecham researchers enhanced the potency of ciglitazone by increasing its lipophilicity. When a urea or thiourea moiety was introduced into the ether side chain, a
tenfold increase in potency was observed. Further modification involved the incorporation of the urea moiety into a heterocyclic ring. Rosiglitazone emerged from this approach. Both it and pioglitazone lower blood sugar levels by reducing peripheral resistance to insulin in patients with type 2 diabetes. They are prescribed in conjunction with another oral hypoglycaemic drug such as metformin or a sulfonlurea.

GUANIDINE AND ITS ANALOGUES

C.K. Watanabe at Yale found that removal of the parathyroid gland resulted in the appearance of abnormal amounts of guanidine in the blood and claimed this was responsible for the drop in blood sugar levels following parathyroidectomy. He also demonstrated in rabbits that blood sugar levels fell after injection of guanidine. When guanidine was tested as a potential antidiabetic agent it proved to be toxic.

Karl Slotta at the Chemistry Institute at the University of Vienna synthesised a series of compounds with guanidine groups at each end of a long polymethylene chain. They were more potent than the earlier monoguanidine compounds and less toxic. Following a clinical trial in 1926, one of these was marketed as Synthalin® by Schering AG of Berlin for use as an oral hypoglycaemic agent in mild cases of diabetes. As a result of adverse reports about Synthalin®, the related Synthalin B® was introduced with the claim that it was safer. However, the high incidence of side effects discouraged diabetics from taking either of these and they were finally withdrawn in the early 1940s because of evidence of liver toxicity.

The introduction of the sulfonylureas as oral antidiabetic agents renewed interest in the guanidines. In 1957, Seymour Shapiro, Vincent Parrino and Louis Freedman of the US Vitamin Corporation in Yonkers, New York, synthesised the biguanide known as ‘metformin’. It was one of more than 200 biguanides that had been examined. Metformin does not act by stimulating the islet cells of the pancreas to produce more insulin, as do the sulfonylureas. Rather, it decreases gluconeogenesis and increases peripheral glucose utilisation.
Evidence obtained in 1928 indicated that trypanosomes required relatively large amounts of glucose in order to reproduce.\textsuperscript{76} Seven years later, Hildrus Poindexter at the Howard University School of Medicine in Washington DC demonstrated that survival of animals infected with trypanosomes was prolonged if their blood glucose levels were kept depressed by insulin injections.\textsuperscript{77} At the University of Szeged, Nikolaus von Jancso then exposed mice infected with \textit{Trypanosoma brucei} to Synthalin\textsuperscript{1} and several of its analogues.\textsuperscript{78} These turned out to have a trypanocidal action. At the Liverpool School of Tropical Medicine, Warrington Yorke and E.M. Lourie were quick to note that Synthalin\textsuperscript{1} killed the trypanosomes at dose levels that did not significantly lower blood sugar in mice. They demonstrated that Synthalin\textsuperscript{1} was even active against trypanosomes growing on culture media rich in glucose. This indicated that the trypanocidal action of the amidines was a direct one, and had nothing to do with lowering blood sugar in the host animal.\textsuperscript{79} The Liverpool findings were then reported to Harold King at the National Institute for Medical Research, which led to his preparing analogues of Synthalin\textsuperscript{1} as potential trypanocidal agents. Among these were several diamidines that turned out to be powerful trypanocides that cured mice and rabbits infected with \textit{T. rhodesiense}. The analogue with one extra methylene group in the chain was found to be particularly active in mice.\textsuperscript{80}

In 1937, Arthur Ewins of May and Baker was invited to participate in the research programme. He arranged for the preparation of diamidines in which the polar amidine groups were separated by an intermediate chain consisting of two benzene rings rather than polymethylene groups, as had previously been the case. The first of his compounds proved to be active, so the series was extended, with a large number of analogues being examined. Many of these were trypanocidal, especially stilbamidine and pentamidine.\textsuperscript{81} As pentamidine had greater water solubility than stilbamidine, it was preferred for intramuscular injections.

![Stilbamidine](image1)

![Pentamidine](image2)

By 1940, the diamidines had been tested on over 400 patients suffering from sleeping sickness or the related tropical disease, leishmaniasis. Because of their polar nature, they were unsuitable for treating advanced forms of sleeping sickness in which there was central nervous system involvement. Nevertheless, these diamidines, prepared as a result of close collaboration between academia and industry, are still used against trypanosomiasis. They are also active against a range of protozoa, including \textit{Pneumocystis carinii}.\textsuperscript{82} Pentamidine isethionate has proved to be a life-saving drug when administered by aerosol into the lungs of AIDS patients infected with this organism.

It has been known since 1938 that amidoximes (i.e. \textit{N}-hydroxyamidines) also have trypanocidal activity.\textsuperscript{83} After reading a report on the activity of some of these compounds, Mull synthesised Su-4029 at the Ciba laboratories in Basle. This proved inactive against trypanosomes, but was found to possess antihypertensive activity in dogs after being routinely submitted to Ciba’s general screening programme.
A clinical trial confirmed the antihypertensive activity of Su-4029, but also revealed that it induced fever. Mull then prepared several analogues.84 One of these was guanethidine, which is still sometimes administered along with a diuretic or beta-blocker in cases of resistant hypertension. It acts both by blocking the release of noradrenaline from postganglionic adrenergic neurones and (unlike its analogues) also depletes the nerve endings of noradrenaline. Adrenergic blockers such as guanethidine and its analogues are no longer popular because they cause postural hypotension. Several analogues of guanethidine were marketed in the mid-1960s. One of these was debrisoquine, which is still occasionally used in combination with a beta-blocker or diuretic in the treatment of hypertension.85

PYROPHOSPHORIC ACID ANALOGUES

At the University of Berne, Herbert Fleisch discovered that plasma, urine and saliva inhibited calcium phosphate precipitation because they contained inorganic pyrophosphate, a substance not previously known to be present in these fluids.86 Pyrophosphate was then shown to reduce both the dissolution of calcium phosphate crystals and also their aggregation into larger clusters.87 When injected into rats, pyrophosphate inhibited experimentally induced deposition of calcium in soft tissue, but had no effect on bone resorption (loss of calcium phosphate).88 This lack of effect on bone resorption was assumed to be due to rapid hydrolysis of pyrophosphate by alkaline phosphatase.

Fleisch prepared biphosphonate analogues of pyrophosphate in which the metabolically sensitive central oxygen atom was isosterically replaced by a carbon atom. Low concentrations of many of these were shown to behave like pyrophosphate by inhibiting precipitation of calcium phosphate and the aggregation of its crystals.89 It was found that this was due to a common affinity for the solid phase of calcium salts, to which they strongly bound.90 The clinical implication was evident since pyrophosphate is present in plasma, teeth and bone. Topical application of the new biphosphonates in rats reduced the formation of dental tartar, which in part consisted of calcium phosphate and carbonate.91 This explains why Procter and Gamble, a company with a major interest in toothpaste formulation, was collaborating with Fleisch. A dental hygiene product was later marketed.

Safety was a paramount factor in selecting a biphosphonate for clinical testing. Among the biphosphonates that had been prepared, the tendency to interfere with normal calcification in bone, cartilage and dentine varied. Although this was reversible, it had to be taken into consideration. After careful evaluation, disodium etidronate was selected for evaluation in the clinic after it had been shown to inhibit hydroxyapatite dissolution in vitro and bone resorption in vivo.92 The prime interest lay in the role of disodium etidionate in Paget's disease, a metabolic disorder involving excessive bone resorption with abnormal calcification and bone formation. When given by mouth, the drug effectively improved or even abolished bone pain and stopped bone resorption and restored serum calcium levels to near-normal values. These
early clinical studies used doses of disodium etidronate that produced abnormal bone mineralisation, which caused deposition of a layer of osteoid tissue on the bones. This led to pain and fractures. Reduction of the dose resulted in some bone resorption, but a balance was achieved by reducing the dosage and administering the drug in six-monthly cycles. Disodium etidronate was then licensed in the United Kingdom for use in Paget’s disease. The license was later extended to include treatment of vertebral osteoporosis and the bone pain and hypercalcaemia caused by malignancies such as breast cancer or multiple myeloma.

Sodium clodronate was prepared at the same time as disodium etidronate. It is somewhat more effective when used in patients who have hypercalcaemia associated with malignancy. However, Fleisch found that lengthening of the carbon skeleton of disodium etidronate and also incorporating a terminal amino group on the carbon chain markedly increased potency.93 Disodium pamidronate was then shown to be more effective than sodium clodronate in the treatment of hypercalcaemia of malignancy, but it could only be administered parenterally as it was poorly absorbed from the gut.

UNDECENOIC ACID (UNDECYLENIC ACID)

Knowing that human sweat had a reputation for inhibiting the growth of fungi, Peck and Rosenfeld tested the antifungal activity of fatty acids present in sweat and claimed that propionic, butyric, lactic and ascorbic acids were of value in the treatment of fungal infections.94 A systematic examination of a wider range of fatty acids by researchers at Johns Hopkins Hospital showed that antifungal activity increased with chain length and was optimal in formulations at pH 5.95 Foley and Lee later demonstrated that the most potent of the fatty acids was undecenoic acid.96 It is used as a topical antifungal agent.

ANGIOTENSIN-CONVERTING ENZYME

In 1898 at the Karolinska Institute, Robert Tigerstedt and his student Per Bergman discovered that a hypertensive agent was present in a saline extract of kidney tissue.97 Named ‘renin’, the physiological significance of this substance became clearer in 1934 after Cleveland physician Harry Goldblatt demonstrated that it was involved in renal hypertension.98 By 1940, it had been established that renin was an enzyme released from the kidney into the blood, where it promoted the formation of a pressor peptide called angiotensin.99 This peptide was shown to exist in two forms, which were then isolated.100,101 Angiotensin I was a decapeptide without pressor activity, whereas angiotensin II was an octapeptide that turned out to be the most potent blood pressure raising substance in the human body. The hydrolysis of angiotensin I into angiotensin II in the plasma was catalysed by a zinc-containing enzyme named angiotensin-converting enzyme (ACE), which was purified in 1956.102
Further investigation into the clinical role of angiotensin II led to the discovery that, in addition to its potent pressor activity, it also acted directly on the adrenals to stimulate release of aldosterone. This produced sodium retention, thereby enhancing the ability of angiotensin II to raise blood pressure.103

ACE Inhibitors

While working in the Institute of Basic Medical Sciences at the Royal College of Surgeons of England in the 1960s, John Vane was investigating the causes of hypertension. When he tested an extract of the venom of the Brazilian arrowhead viper, Bothrops jararaca, which had been brought to London by Sergio Ferreira, one of his post-doctoral students, he discovered that it could block the formation of angiotensin II from angiotensin I by inhibiting ACE.104 Despite leading clinicians not sharing his opinion, Vane was able to convince researchers at the Squibb Institute for Medical Research in New Jersey that an ACE inhibitor offered a novel approach to the control of high blood pressure. Miguel Ondetti and his colleagues then fractionated the viper venom and elucidated the structure of several peptides.105 One of the most active at inhibiting ACE was teprotide, which had already been isolated in Vane’s laboratory.106 It was now synthesised and investigated thoroughly in animals by the Squibb researchers. The first clinical study showed that when teprotide was administered intravenously in patients with elevated plasma renin levels, it was an effective hypotensive agent.107 Further studies revealed that it has similar activity in hypertensive patients with normal renin levels. As was to be expected with any peptide, teprotide was inactive by mouth. This ruled out any likelihood of its routine use in the clinic as a hypotensive drug, but pointed the way forward for an attempt to synthesise an orally active analogue.

The Squibb research team next screened about 2000 non-peptides in a vain attempt to find an orally active ACE inhibitor suitable for clinical evaluation. A new approach was then taken after the publication of a paper by Byers and Wolfenden about the inhibition by benzylsuccinic acid of another zinc-containing metallopeptidase, the digestive enzyme carboxypeptidase A.108 Byers and Wolfenden had found that natural substrates were bound to the active site of carboxypeptidase A by their C-terminal phenylalanine residue. As benzylsuccinic acid had a similar enough structure to phenylalanine it bound to the enzyme active site, but since it had no amide function that could be hydrolysed it remained bound to the active site, thereby blocking access to it by the natural substrate.

The Squibb researchers knew that hydrolysis of angiotensin I involved the splitting off of a dipeptide rather than a single C-terminal amino acid as in the phenylalanine bound to carboxypeptidase A. This meant that in an inhibitor of ACE, binding to the active site would only occur if the distance between the amino acid moiety and the second carboxylic acid group was extended. Since it was already known that peptide inhibitors with an alanylproline residue
at the C-terminal end had the greatest affinity for the active site of ACE, the Squibb team synthesised succinoyl-L-proline and tested its binding affinity for ACE. This confirmed that succinoyl-L-proline was a specific inhibitor, albeit with low potency. This was enhanced by introducing a methyl substituent adjacent to the amide function, with the appropriate stereochemistry to maintain similarity with the peptide inhibitors. The resulting compound, D-2-methylsuccinoyl-L-proline, was still not potent enough to be considered as a candidate compound for clinical investigations and it required considerable effort and ingenuity to enhance its potency.

The Squibb researchers believed that interaction between the carboxyl group in the carboxyalkanoyl moiety and the zinc atom in the enzyme active site could be increased, but this proved difficult to achieve until its carboxyl was replaced with a thiol group. This resulted in a one-thousand-fold increase in inhibitory activity for captopril. This was the first non-peptide ACE inhibitor suitable for introduction into the clinic. When it was marketed in the United Kingdom in 1981, the Committee on Safety of Medicines limited the product licence to use only in patients with severe hypertension who had not responded to standard therapy. This limitation was imposed because early clinical trials had shown captopril to have the potential to cause renal damage or granulocytopenia. After intensive postmarketing surveillance revealed that side effects were mainly of a minor nature and of relatively low incidence, the licence was extended in 1985, to cover its use in the treatment of mild to moderate hypertension. Captopril and other ACE inhibitors are now firmly established in the treatment of heart failure and in hypertension when thiazide diuretics or beta-blockers are either ineffective or not tolerated.

Investigators at Merck reconsidered the use of succinoyl-L-proline as a non-hydrolysable substitute for the alanylproline residue and examined derivatives of glutamyl-L-proline. This led to the discovery of high activity in compound I.
Taking into account what was known about the specificity of the active site of ACE, the Merck researchers introduced hydrophobic amino acid side chains in place of one of the methyl groups in compound I. Of these, phenylalkyl substituents were found to increase inhibitory activity. Maximal activity occurred in enalaprilat, which was 2000 times more potent than compound I. When administered intravenously, it was more potent than captopril. However, oral bioavailability was disappointing. This was overcome by masking the carboxyl group through conversion to its ethyl ester to form enalapril.110 This was well absorbed from the gut before undergoing hydrolysis in the liver to release enalaprilat.

Merck scientists also decided to determine whether or not the best substrates for ACE were those based on the alanylproline residue at the C-terminal end of peptide ACE inhibitors. Accordingly they examined a variety of dipeptides containing either alanine or proline connected to a variant amino acid. After their results had confirmed the importance of retaining proline at the C-terminal, it was established that lysine could replace the alanine in enalaprilat. The resulting compound, lisinopril, was unexpectedly found to be well absorbed from the gut, unlike enalaprilat. It seems likely that the presence of the lysine residue in lisinopril permits peptide-carrier-mediated transport from the gut into the portal circulation.111

Squibb investigators established that the potent zinc-binding ligand phosphonic acid could be incorporated into ACE inhibitors. In addition, they were aware that the lipophilicity of the proline ring could be increased with retention of activity. They therefore synthesised fosinopril in 1982.112 It was found to be different to other ACE inhibitors insofar as, after being hydrolysed to the active fosinoprilat, it was eliminated roughly equally by both the liver and the kidneys. This meant that it was suitable for administration to patients with reduced renal function.

Several companies independently discovered that the proline ring in ACE inhibitors could be enlarged without any loss of activity. This led to the introduction of quinapril,113 ramipril114 and perindopril.115
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51. W. Sahli, *Arch. Physiol.*., 1886; **38**: 35.
64. Ger. Pat. 1969; 1925423 (to Parke, Davis).


In 1877, Louis Pasteur and Jules Joubert showed that animals inoculated with a mixture of anthrax bacilli and common bacteria did not contract anthrax. Pasteur went on to suggest that microbes liberated materials that might be exploited therapeutically and soon after the Viennese pathologist Victor Babes detected the secretion of substances from one bacterial species that could kill those of another. Ten years later, Garré demonstrated an antibiotic effect arising from diffusion when two different species of bacteria were streaked closely in parallel across an agar plate. That same year, Rudolf Emmerich was giving a demonstration to his class at the University of Munich when he found that a guinea pig he had inoculated with *Vibrio cholera* did not develop cholera. It transpired that the animal had previously been injected with *Streptococcus erysipelatis*. He followed this up by showing that anthrax could be experimentally prevented through prior inoculation with a culture of *Bacillus anthracis*.

**PYOCYANASE**

Eduard von Freudenreich reported from the Pasteur Institute in 1888 that typhoid bacilli often failed to grow in filtered broths in which bacteria had previously been cultured. He went on to demonstrate that *Pseudomonas aeruginosa* (then known as *Bacillus pyocyaneus*) was particularly effective in antagonising the growth of typhoid bacilli and some other bacteria. The following year, Bouchard showed that inoculation of rabbits with *P. aeruginosa* protected them against anthrax. Ten years later, Rudolf Emmerich and Oscar Löw began treating hundreds of patients with pyocyanase, a powdered material that they mistakenly believed to be an enzyme. It was probably obtained by allowing cultures of *P. aeruginosa* to incubate for six weeks, during which time the bacteria decomposed and liberated the antibiotic into solution. Emmerich never disclosed how he prepared his pyocyanase, so most subsequent workers had to purchase commercially prepared material. It was claimed that this destroyed a variety of pathogenic bacteria, including the causative organisms of diphtheria, anthrax, plague and typhoid. Although the therapeutic use of pyocyanase became a controversial matter, its use continued until 1913 when the commercial product suddenly ceased to have any activity. Investigations continued and it was found that the active material was highly lipophilic in nature. Edward Doisy and his colleagues at St Louis University School of Medicine in 1945 isolated five lipophilic antibacterial substances from cultures of *P. aeruginosa* active mainly against Gram-negative bacteria. Three of them were subsequently identified by Ibert Wells at Syracuse University, New York, as 2-heptyl, 2-nonyl and 2-(Δ1-nonenyl)-4-hydroxyquinoline. The arrival of penicillin meant that there was no further interest in them.

**TYROTHRICIN**

At the Rockefeller Institute in New York in 1939, Rene Dubos isolated a bactericidal, protein-free extract from *Bacillus brevis*. This material, tyrothricin, was soon shown to consist mainly
of a cyclic decapeptide called tyrocidin, together with a similar compound called gramicidin S, which was 50 times as potent.\textsuperscript{12} Their structures were elucidated by Richard Synge at the Lister Institute in London using paper chromatography in one of its earliest applications.\textsuperscript{13,14} Although both components were able to protect mice against pneumococci, they were too toxic for general use.

Tyrothricin was suitable only for topical application. It was marketed in the USA by Sharp & Dohme in 1942 for treatment of Gram-positive infections. Since then, it has been universally used in throat lozenges as a non-prescription antibiotic, but the commercial success of this type of product has been largely due to the incorporation of benzocaine, a topical anaesthetic that soothes sore throats.

FUNGAL ANTIBIOTICS

The first scientific report of the antimicrobial activity of the familiar green \textit{Penicillium} mould found on oranges or jam appeared in 1870. John Burdon-Sanderson of St Mary’s Hospital in London, who was one of the first British physicians to accept Pasteur’s ideas on the germ theory of disease, noted that bacteria did not grow and produce turbidity in sterilised culture solutions that had become contaminated by an air-borne \textit{Penicillium} mould.\textsuperscript{15} A series of experiments confirmed this, although Burdon-Sanderson had misinterpreted his observations by concluding that only fungi, and not bacteria, could cause aerial contamination of culture solutions.\textsuperscript{16}

In January 1895, Vicenzo Tiberio of the Sanatorio Militare Marittimo Hospital in Naples published an account of work on the antibacterial properties of moulds.\textsuperscript{17} An extract of a mould that he identified as \textit{P. glaucum} had inhibited the growth of staphylococci and other pathogenic bacteria. He gave a detailed description of its action on infected rabbits, guinea pigs and rats. The following year, his countryman Bartolomeo Gosio isolated a crystalline product from a mould that he thought was \textit{P. glaucum}.\textsuperscript{18} There was no connection between these two investigations in Italy, for Gosio had been examining fungal growth on spoiled maize in an attempt to identify the cause of pellagra. Nevertheless, since the chemical properties of the crystalline compound indicated it was a phenol, he tested its antiseptic properties on cultures of the anthrax organism. This showed it to be an inhibitor of their growth. Lack of material prevented him from carrying out animal experiments, but the phenolic crystalline antibiotic was named ‘mycopenolic acid’ in 1913. It seems probable that the early claims of antibacterial activity among varieties of \textit{Penicillium} moulds relate to this compound, the species described as \textit{P. glaucum} most likely really being \textit{P. brevit Compactum}.
Several more reports of antibacterial activity associated with *P. glaucum* were published before mycophenolic acid was investigated by Harold Raistrick at the Department of Biochemistry at the London School of Hygiene and Tropical Medicine in the early 1930s. Its structure was elucidated in 1952, and found to be chemically unrelated to benzylpenicillin.\textsuperscript{19} Although mycophenolic acid was too toxic for clinical application as an antibiotic, in 1969 it was shown to be a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), causing cessation of purine synthesis.\textsuperscript{20} This led to its clinical evaluation as an immunosuppressant in transplant surgery\textsuperscript{21} and psoriasis.\textsuperscript{22} When taken by mouth, the morpholinoethyl ester, known as ‘mycophenolate mometil’, had enhanced bioavailability.\textsuperscript{23} It is a prodrug that releases mycophenolic acid after absorption from the gut. It has become accepted as a useful immunosuppressant.

**Penicillins**

Alexander Fleming, director of the Inoculation Department at St Mary’s Hospital in Paddington, London, was extremely fortunate when a remarkable combination of events occurred in the summer of 1928 and permitted him to discover the antibacterial activity of penicillin. The probable sequence of these events has been reconstructed in an account written by his former assistant, Ronald Hare.\textsuperscript{24} Firstly, on the floor beneath Fleming’s laboratory a colleague worked with moulds required for the production of vaccines to treat allergies, and it seems likely that one of these was wafted through the air into Fleming’s laboratory to settle on a petri dish covered with a layer of agar impregnated with staphylococci. Secondly, this mould was a rare strain of *Penicillium notatum* that produced significant amounts of penicillin. Thirdly, Fleming left his culture plate on his work bench instead of placing it in an incubator at body temperature to ensure bacterial growth. Fourthly, an exceptionally cool spell followed when Fleming went on holiday at the end of July, which favoured growth of the mould in preference to that of the staphylococci. Fifthly, the climatic conditions changed later in the month, by which time the mould had produced sufficient penicillin to kill bacteria in its vicinity. This rise in temperature allowed colonies of staphylococci to grow elsewhere on the culture plate, thus enabling Fleming to observe a zone of inhibition of staphylococcal growth when he returned to the laboratory on 3 September. The original plate is kept in the British Museum, Fleming having treated it with formaldehyde vapour to preserve it.

As a leading authority on antiseptics, Fleming was ideally qualified to make a realistic assessment of the significance of the effect of the mould on bacterial growth. He prepared subcultures of the mould and gave the name ‘penicillin’ to the filtrate of the broth in which these had been grown for one or two weeks at room temperature. Two assistants, Frederick Ridley and Stuart Craddock, were given the task of preparing sufficient quantities of penicillin for bacteriological studies. They were also asked to obtain information about the chemical nature of the active substance, this initially being assumed to be an enzyme. They devised an efficient process for growing the mould in large, flat-sided bottles from which the juice below the surface growth was drained and filtered. As boiling destroyed the activity of the mould juice, acidification and evaporation at 4°C under reduced pressure was used. The resulting concentrate was taken up in alcohol, in the process of which much of it was precipitated. The

\[
\text{\begin{align*}
\text{CH}_3 & \quad \text{O} \\
\text{N} & \quad \text{O} \\
\text{CH}_3 & \quad \text{CO} \\
\text{O} & \quad \text{H}_3C \\
\text{CH}_3 & \quad \text{OH} \\
\end{align*}}
\]

\textit{mycophenolate mometil}
activity was found in the alcohol, ruling out the likelihood of penicillin being an enzyme. The alcohol extract was quite potent, but its activity gradually disappeared over a period of weeks. This later discouraged Fleming from pursuing the therapeutic possibilities of penicillin.

Fleming carried out tests to find which types of bacteria were sensitive to the penicillin extract. He established that it could lyse a variety of major pathogens, but the marked insensitivity of *Haemophilus influenzae* to it particularly interested Fleming. This had proved to be a difficult organism to isolate from infected patients since cultures of it were readily overgrown by other bacteria, preventing the preparation of vaccines. At that time, many believed (wrongly) that this organism was the cause of influenza and other respiratory conditions. In an attempt to kill off interfering bacteria, Fleming incorporated penicillin in cultures of *H. influenzae* prepared from throat swabs. His ploy worked admirably, and for the next ten years or so was the principal purpose for which penicillin was used. Indeed, the title of Fleming’s first paper on penicillin was ‘Cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*’.25

In December 1928, Fleming examined the effect of penicillin on slides of bacteria growing in the presence of blood or serum, in the presence of which practically every known antiseptic had been rendered worthless, other than for topical application where sufficiently high concentrations could be achieved. When this test indicated that the activity of penicillin was diminished, he no longer expected it to be active against generalised infections or those in deep wounds. He subsequently learned from Craddock that penicillin injected into a rabbit disappeared from its blood within 30 minutes, which was most discouraging as it had been established that penicillin required around four hours to act. Fleming went on to investigate the potential of penicillin as an antiseptic for topical application. He began by treating Craddock’s infected nasal antrum with penicillin, but to no avail. A second disappointment followed when it failed to cure an infected amputation stump and the patient died from septicaemia. Fleming did have one success with penicillin when he applied it to the eye of another of his assistants who had contracted a pneumococcal conjunctivitis. This time the infection rapidly cleared.

At the beginning of April 1929, Fleming and Craddock incubated the organs of a newly killed rabbit in a liquid culture of staphylococci for 24 hours and then transferred them to a penicillin solution for a similar period. Examination of slices of the organs revealed that staphylococci which penetrated deep within the organs had survived. It was concluded that penicillin could not penetrate beyond the surface of organs. This was a misleading interpretation since it failed to consider the possibility of circulating blood being able to carry penicillin into the tissues. It cast further doubt on the clinical value of penicillin. Unfortunately, Fleming never administered penicillin to infected mice and it was left to others to discover its outstanding therapeutic efficacy. When he did inject penicillin into a rabbit and a mouse, it was solely to confirm its lack of toxicity.

After submitting his paper to the *British Journal of Experimental Pathology*, Fleming carried out little further research on the clinical potential of penicillin other than occasionally applying it locally to treat infections. His subsequent researches with it were mainly as a reagent in bacteriological investigations. As a biographer of Fleming has pointed out, this ensured that cultures of the original strain of *P. notatum* were available when requested by laboratories, including that of the School of Pathology at Oxford.26

Harold Raistrick at the London School of Hygiene and Tropical Medicine assigned Percival Clutterbuck to the isolation of penicillin. The bacteriological assays were carried out by Reginald Lovell. Even though Raistrick spoke to Fleming on the telephone several times, he was never told about Ridley and Craddock’s isolation procedure. Eventually, an ether extract of penicillin was obtained, but evaporation of the solvent resulted in the loss of its activity.27 Baffled by this, Raistrick abandoned the project since there was no good reason to deploy the extensive resources that would be required to pursue the investigation further.
The next attempt to purify penicillin came from Fleming’s laboratory in 1934. By now Ridley and Craddock had both left the Inoculation Department, but Holt, a chemist, had joined the staff. He made quick progress, thanks to Clutterbuck’s discovery that penicillin could be extracted into organic solvents from slightly acid solutions. This enabled him to recover penicillin from an amyl acetate extract by partitioning it with a very slightly alkaline solution, a procedure that had not been tried by anyone else. The instability of penicillin in this solution led him to abandon his work on it after only a few weeks, without publishing his findings.

In the summer of 1938 at the Sir William Dunn School of Pathology at Oxford, Ernst Chain became interested in naturally occurring antibacterial substances. Penicillin was one of three substances that particularly interested him as it seemed likely to be an enzyme. Through a stroke of good fortune he then encountered a colleague carrying a mould dish along a corridor and learnt that it contained penicillin supplied by Fleming. He now believed a detailed study of how it lysed the bacterial cell wall would afford useful information on the structure of this wall, but he had no reason to believe that it would be of any particular therapeutic value. He was encouraged to pursue this idea by Howard Florey, an Australian who held the Chair of Pathology at Oxford. Florey had encountered penicillin in 1932, when a colleague employed it in three cases of skin infections. Florey, Chain and their colleagues published an account of the subsequent work on penicillin at Oxford in 1949. Since then, books by Hare, Macfarlane, Clarke and others have enabled a detailed picture to be assembled about the momentous events that were to be of so much benefit to mankind, yet which took place during the darkest days of Western civilisation as war again swept across Europe.

Chain used a culture of Fleming’s original mould provided by a colleague who had propagated it at Oxford for several years after it had been obtained for use as a bacteriological reagent. Unaware of the previous work by Holt, Chain rediscovered the solvent extraction procedure. While doing this, he realised that the physical properties of penicillin indicated that it could not be an enzyme as he had anticipated. It seemed to be a small molecule.

Work reported in 1939 from the Rockefeller Institute in New York on tyrothricin gave an added dimension to the research on penicillin. With Chain’s research grant about to expire, Florey now sought financial support for the penicillin project on the grounds that penicillin was active against pathogenic bacteria. He argued that since previous workers had used only crude penicillin, every effort should be made to obtain it in pure form so that the effects of injecting it intravenously could be assessed. Thus the ultimate discovery of the value of penicillin actually depended upon that of an earlier antibiotic of questionable therapeutic benefit – and an inspired Australian!

In October 1939, the Medical Research Council awarded Chain an annual grant of £300 plus £100 for materials, this to be for each of three years. Shortly after, the Rockefeller Foundation awarded Florey a magnificent annual grant of $5000 for five years, plus an initial sum to purchase equipment. This large sum guaranteed the viability of the project and must rank as the finest investment ever made by a charitable foundation. Of course, it had also been the work on tyrothricin at the Rockefeller Institute that led to the potential clinical role of penicillin being evaluated. This transatlantic co-operation was soon to progress much further.

The Rockefeller Foundation also awarded a fellowship to Norman Heatley, whose ability to improvise apparatus for the large-scale production of penicillin was to prove invaluable. With support for the project secured, work on penicillin proceeded at a fast pace under Florey’s skilled administration, with the entire staff of the School of Pathology becoming involved. Heatley put considerable effort into finding the best means of producing mould juice and recovering active material from it. It was not, however, until 1941 that Chain established the superiority of amyl acetate as an extraction solvent; this had also been previously discovered by Holt. This solvent then replaced ether in Heatley’s mechanised countercurrent extraction system, which involved transference of penicillin in downward-flowing streams of filtered,
acidified mould juice into upward-flowing streams of the immiscible solvent in the same glass tubes. A reversal of the procedure was utilised for the back-transfer of the penicillin to slightly alkaline, aqueous solution. Recovery of penicillin from this had thwarted Holt in 1934, but the following year the process of freeze-drying had been developed in Sweden and was now introduced at Oxford. It enabled Heatley to recover a brown, dry powder containing around 5 Oxford units per mg, which, although far from pure, was suitable for biological studies. By mid-March 1940, Chain had in his possession a supply of about 100 mg of this powder. A substantial portion was then injected intraperitoneally into two mice, with no ill effects being observed. From here on, Florey took over the complex biological, toxicological and clinical investigations, gambling the entire resources of his department on the production and purification of penicillin, while having no proof that it would be effective when injected. It had only been proven to be a local antiseptic and practically all antiseptics were ineffective when injected. Neither Fleming, Raistrick nor Holt had ventured further, but a major development had occurred since they worked on penicillin. In 1935, I.G. Farben introduced Prontosil, the synthetic antibacterial sulfonamide that was the first drug ever to cure systemic infections. Its success had revolutionised medical thinking, else Florey would never have seriously considered proceeding as he did.

The crucial experiment with penicillin took place on Saturday 25 May 1940. At 11 am, Florey injected each of eight mice with a lethal amount of virulent streptococci, a technique used by Gerhardt Domagk during the development of Prontosil. At noon, two mice were injected subcutaneously with 10 mg of penicillin and two others received half this amount. These latter two received four more similar injections during the next ten hours. Heatley stayed in the laboratory that night and watched the untreated mice die. One that received a single dose of penicillin died two days later, while all the others survived. This greatly encouraged all those engaged on the project, and they proceeded to conduct extensive biological studies with the crude powder. In July, it was confirmed that penicillin was also effective in mice infected with either staphylococci or Clostridium septicum, the causative organism of gas gangrene. The results of the investigations were published in the Lancet on 24 August 1940. Only brief details were given, but the fact that it appeared at all is evidence of the lack of importance attached to penicillin in comparison with projects considered to be of military importance, such as a major one concerning antimalarials. Ten days after this paper appeared Fleming visited Oxford to make his first contact with Florey’s department. Florey must have been disappointed that this was the sole response to his paper, for he had hoped that publication of his preliminary findings might impress the pharmaceutical industry. Earlier that summer, he had approached Burroughs Wellcome to ask if they would produce penicillin on a large enough scale for him to begin clinical trials. This offer was declined because their facilities were stretched to the limit in trying to prepare blood plasma and also meet the requirements of the armed forces for vaccines. It was felt that penicillin would not be as important for the war effort.

By January 1941, the School of Pathology had become a factory for the production of penicillin. Chain and Abraham now tried the new technique of adsorption chromatography, which enabled penicillin to be adsorbed on to a column of powdered alumina after a solution of it was poured in. The impurities passed down through the column with the solvent, but penicillin was retained on the alumina. It was recovered by solvent extraction of the alumina, the material obtained in this manner having an activity of 50 units per mg. As a result of this development, a single intravenous injection of 100 mg of penicillin was administered to a woman dying of cancer. The sole adverse reaction was a bout of shivering followed by a fever, which was due to impurities that were then removed by chromatography. Further studies on volunteers provided valuable information about the best way of administering penicillin. For example, it was learned that penicillin was destroyed by the acidity of gastric juice, so could not be given by mouth. Since the kidneys were found to excrete penicillin rapidly from the
body, it had to be given by slow intravenous drip in order to maintain adequate bactericidal levels in the body.

The first attempt to treat a patient with a life-threatening infection took place at the Radcliffe Infirmary, Oxford, on 12 February 1941. The patient was a 43 year old policeman with a mixed staphylococcal and streptococcal infection that had spread throughout his body and had already necessitated removal of his eye. He had not responded to sulfapyridine, yet only 24 hours after receiving 200 mg of penicillin intravenously, followed by 100 mg every three hours, his condition improved rapidly. On the third day of treatment, the supply of penicillin ran out. More was obtained from the School of Pathology, where all the urine voided by the patient had been extracted to recover the penicillin. By this expedient, it proved possible to continue the injections for a further three days. The policeman’s health remained good for the next ten days, but a residual lung infection then flared up and he died on 15 March. Before his death occurred, two other patients responded to penicillin treatment. The fourth was a child with a severe infection behind the eye. This disappeared after large doses of penicillin were administered, but the child died when an artery in the brain ruptured as a result of damage caused prior to treatment with penicillin. Two other very ill patients recovered.

The second paper on penicillin appeared in the *Lancet* on 16 August 1941.33 It gave details of penicillin production, animal results and clinical reports. By the time it was published, Florey and Heatley were in the United States seeking to arrange large-scale production of penicillin for extensive clinical trials.34 Before departing, Florey had approached the Boots Company and ICI, but nothing had come of this. When the Rockefeller Foundation offered to pay the expenses for Florey and a colleague to visit the United States to discuss penicillin production, Florey referred the matter to the secretary of the Medical Research Council. He was advised to proceed with the visit as no British manufacturer was in a position to produce penicillin because of the wartime situation.

Soon after their arrival in the United States, Florey and Heatley met Charles Thom at the US Department of Agriculture, who told them that the only way of producing large quantities of penicillin was in deep fermentation tanks similar to those used by brewers. He put Florey in touch with Robert Coghill, head of the fermentation division at the Department of Agriculture’s Northern Regional Research Laboratory in Peoria, Illinois. Coghill agreed to attempt to grow the *Penicillium* in deep culture tanks if Heatley would work on the project for several months. Production of penicillin began the following day, using a culture Florey had brought from Oxford. The yield of penicillin was increased twelvefold within six weeks by including corn steep liquor in the culture medium. This was a syrupy waste product from the manufacture of corn starch. A further improvement was strict control of the acidity of the culture to minimise penicillin degradation.

A few weeks after arriving in the United States, Florey met the research directors of Merck and Company, E.R. Squibb and Sons, Charles Pfizer and Company and Lederle Laboratories. His mission was greatly assisted by the presentation of a paper on penicillin a few months earlier by Martin Dawson, Karl Meyer and Gladys Hobby of Columbia University College of Physicians and Surgeons. This had been given at the annual conference of the American Society for Clinical Investigation, an abstract being published in their widely read journal.35 The paper described how the authors had grown cultures of penicillin obtained from the Oxford team and briefly outlined clinical results on testing a crude preparation on patients. Several American companies had also conducted exploratory work on penicillin before the arrival of Florey and Heatley.36

Merck agreed to proceed with penicillin production at once and also to exchange information with other interested parties, but the other companies reserved their position. After further meetings, Squibb and Pfizer joined in the collaborative effort. Another consortium calling itself the Midwest Group was formed when Abbott Laboratories, Eli Lilly
and Company, Parke, Davis and Company and the Upjohn Company also agreed to exchange information on penicillin. Wyeth Laboratories took up penicillin production near Philadelphia by growing the mould in cellars where mushrooms had previously been cultivated for the gourmet market. They became the largest producer of penicillin until deep fermentation processes were introduced.

The first large-scale clinical use of penicillin in America was unrehearsed. On the night of 28 November 1942, over 500 people perished in a disastrous fire at the Coconut Grove night club in Boston. As soon as it was known that there were 220 badly burned casualties, the Committee on Medical Research authorised the release of supplies of penicillin in an attempt to reduce the anticipated mortality among the survivors. The drug exceeded all expectations, but the public were not told since penicillin was classified as a US military secret.

At Peoria, intensive effort was put into finding a strain of the *Penicillium* mould that would grow in deep tanks and also deliver a higher yield of penicillin. The US Army Transport Command delivered thousands of samples from all over the world, either in the form of soil or as cultures of soil organisms. Despite this effort, one of the best improvements in penicillin yield was obtained with a strain of *P. chrysogenum* growing on a cantaloup melon from the fruit market in Peoria! As part of a collaborative effort involving Minnesota, Stanford and Wisconsin Universities, as well as the Carnegie Institution at Cold Spring Harbor, this strain was irradiated with X-rays, producing mutants that provided even higher yields of penicillin. At the University of Wisconsin, one of the tens of thousands that were to be examined was found to produce 500 units of penicillin per millilitre. This became the standard strain for wartime production of penicillin in America.

It took two years of intense effort before large amounts of penicillin could be produced in deep fermentation tanks containing 12,000 gallons of mould juice. When this was eventually achieved, the impact was staggering. During the first five months of 1943, American manufacturers had delivered 400 million units of penicillin, but during the next seven months 20,000 million units were produced by deep culture. In January 1944, Charles Pfizer and Company alone prepared 4000 million units, yet by the end of that year they had become the world’s largest producer of penicillin, turning out 100,000 million units a month! When the invasion of occupied France began in June 1944, enough penicillin was available to satisfy all military requirements.

Following approaches from Fleming, the British Ministry of Supply established a Penicillin Chemical Committee late in 1942 to co-ordinate industrial manufacture. Boots, Burroughs Wellcome, Glaxo, ICI, Kemball Bishop and May and Baker eventually produced penicillin using the surface culture method. Nevertheless, when in March 1943 Florey and his wife published the results in 187 cases, most of the penicillin had been made at Oxford. Only enough had been available to treat 17 of these patients by intravenous injection; the others received the drug by direct local application. Several pharmaceutical companies in both Britain and the United States had been reluctant to commit themselves to the production of penicillin by a fermentation process. As recently as 1938, the Lederle division of the American Cyanamid Company had lost millions of dollars when a plant for the production of pneumonia vaccines was rendered obsolete in only eight months by the introduction of sulfapyridine. In the 1940s there was a general expectation that penicillin would shortly be synthesised and produced on as large a scale as the sulfonamides. In the event this was not to be so and Merck, the company that made the largest commitment to penicillin synthesis, lost out to rivals who stuck to fermentation processes.

Preliminary work towards determination of the chemical structure of penicillin was initiated when Robert Robinson and his colleagues at the Dyson Perrins chemistry laboratory at Oxford joined forces with Florey’s group early in 1942. Their first investigations had to be carried out with penicillin that was only about 50% pure. Further purification by means of crystallisation was essential if the structure was to be determined. Oskar Wintersteiner and his
group at the Squibb Laboratories achieved this in the summer of 1943. After the news of this reached Oxford, Florey’s group managed to crystallise their penicillin, only to find it was different to that isolated in the United States. This led to the realisation that there were variant forms of the antibiotic, with different side chains. The Oxford material was named penicillin F (it was shown to be 2-pentenylpenicillin). The American product obtained from deep fermentation was designated as penicillin G. This was the one that came into routine clinical use, later receiving the approved name of ‘benzylpenicillin’. Five more variant penicillins were identified the following year.

The Committee on Medical Research set up a project to deal with penicillin synthesis, sponsoring research in American universities, independent foundations and industrial laboratories. Roger Adams of the University of Illinois was in overall charge. In the United Kingdom, the Committee for Penicillin Synthesis was established, permitting a formal exchange of information between British and American scientists while maintaining strict secrecy about the chemical nature of penicillin.37

By late 1943 chemists at Oxford and the Merck laboratories had concluded that penicillin had one of two possible chemical structures. One was a five-membered oxazolone ring joined to a thiazolidine ring, while the other had a rare four-membered beta-lactam ring fused at two points to the thiazolidine ring. Attempts were made to synthesise each of these compounds, with more than a thousand chemists in 39 university and industrial laboratories being involved. Most of their efforts were in vain, but the Merck and Oxford groups did obtain trace amounts of synthetic penicillin. This did not settle the issue of which chemical structure was correct, as the possibility of interconversion existed. This was resolved in 1945 when Dorothy Hodgkin at Oxford employed X-ray crystallographic studies to confirm that penicillin contained the beta-lactam ring system.38 However, with the ending of the war and the winding up of the collaborative programmes, all the laboratories decided to abandon the synthetic work.39

In 1948, benzylpenicillin was formulated as its sparingly soluble procaine salt for use as a depot intramuscular injection.40 It provided effective levels in the tissues for up to 24 hours and became the favoured form of penicillin for the treatment of syphilis, rendering the arsenicals obsolete.

Cephalosporins

Following the clinical introduction of penicillin, Guiseppe Brotzu, the director of the Instituto d'Igiene in Cagliari, Sardinia, began to search for antibiotic-producing organisms. Believing that the self-purification of sea water near a local sewer might be due in some measure to microbial antagonism, Brotzu sampled the water and isolated a mould, Cephalosporium acremonium, which inhibited the growth of typhoid bacilli and other pathogens growing on agar plates. He then prepared hundreds of subcultures of the mould until he had isolated a strain that conferred high antibacterial activity on filtrates of mould juice. Adding alcohol to an extract from the juice precipitated inactive material, leaving a solution that had a wider spectrum of antibacterial action than penicillin and active against Gram-negative as well as Gram-positive bacteria. When preliminary clinical studies were carried out by direct
application of filtered mould juice to boils and abscesses caused by staphylococci and streptococci, the results were most encouraging. The concentrated extract was then injected into patients with typhoid, paratyphoid and brucellosis, again with good results. Brotzu published his findings in a pamphlet describing the work being conducted at the Instituto d’Igiene.\(^{41}\) This did not receive a wide circulation, but a copy was passed to Florey, resulting in a culture of *Cephalosporium* being sent to Oxford in September 1948.

Florey made arrangements for the mould to be grown in deep culture tanks at the Medical Research Council’s Antibiotic Research Station at Clevedon in Somerset. At Oxford, Heatley then extracted an acidic antibiotic from the *Cephalosporium* mould juice. By July 1949, Edward Abraham and H.S. Burton\(^{42}\) had isolated an antibiotic from Heatley’s solvent extract, but were disappointed to find that it was active only against Gram-positive bacteria. Accordingly, they designated it ‘cephalosporin P’. Its chemical structure was not established until 1966, after it had been shown to consist of at least five components, of which the major one was designated cephalosporin P\(_1\).\(^{43}\)

![Cephalosporin P\(_1\) and Penicillin N](image)

A second antibiotic produced by *Cephalosporium* spp. growing in sewage was detected in mould juice that had already been extracted with organic solvent.\(^{44}\) Since this had the activity of Brotzu’s original material, it was named cephalosporin N, reflecting its activity against Gram-negative as well as Gram-positive organisms. It proved difficult to isolate, but Abraham and Guy Newton showed it to be an unstable penicillin and renamed it penicillin N.\(^{45}\) Shortly after, Newton assisted the staff of the Antibiotic Research Station to increase the yield of the antibiotic, thus finally permitting its isolation in fairly pure form by Abraham in 1955.\(^{46}\) It turned out to have only a hundredth of the activity of benzylpenicillin against Gram-positive bacteria, but was much more active against Gram-negative organisms. It now became evident, for the first time, that the nature of the side chain in a penicillin could have a marked effect on the spectrum of antibacterial activity. In the late 1950s, Abbott Laboratories prepared sufficient supplies of penicillin N for its clinical value in typhoid to be established. The results were satisfactory, but the introduction of the semi-synthetic penicillins rendered it obsolete.

While completing degradation studies to confirm their proposed chemical structure for penicillin N, Newton and Abraham separated three contaminants from a crude sample of the antibiotic. The third of these was isolated as its crystalline sodium salt. Surprisingly, it exhibited weak antibiotic activity, and so was given the name ‘cephalosporin C’. Abraham and soon Newton discovered that it was chemically related to benzylpenicillin, but had a much greater ability to withstand the destructive action of beta-lactamases produced by bacteria. It was also less toxic, holding out the promise of being able to inject large doses to destroy staphylococci that had become resistant to penicillin by producing beta-lactamases.

Major problems had to be overcome before cephalosporin C could be produced on a scale large enough to meet the anticipated demand. These were overcome at the Antibiotic Research Station. Patents on cephalosporin C production were taken out by the National Research Development Corporation, which had been set up in 1949 to exploit discoveries from British universities and government laboratories.\(^{47}\) Licences were issued to Glaxo Laboratories and several foreign manufacturers. These provided a rich dividend for the British taxpayer when
cephalosporin C later became the principal starting material for the production of semi-synthetic cephalosporins.\(^4\)

Plans to market cephalosporin C had to be scrapped in 1960 when Beecham Research Laboratories introduced methicillin, a penicillin that was resistant to beta-lactamases. The following year, Abraham and Newton elucidated its chemical structure.\(^4\) Like benzylpenicillin, it possessed a sensitive beta-lactam ring, but fused to a dihydrothiazine rather than a thiazolidine ring. The future of the cephalosporins was assured when it was discovered that superior analogues could be prepared in much the same way as was to happen with the penicillins.

Cephamycin C was first isolated from *Streptomyces clavuligerus* at the Merck Sharpe & Dohme Research Laboratories in 1971.\(^5\) It became the most widely studied of the natural cephamycins since it was resistant to beta-lactamases. This enhanced its activity against certain Gram-negative organisms (other than pseudomonal strains) that were not susceptible to cephalosporins, though it lacked activity against Gram-positive bacteria.

**Griseofulvin**

The failure of newly planted conifers to grow on Wareham Heath in Dorset during the 1930s was attributed to the presence in the soil of a substance toxic to the fungi whose presence was essential for normal tree development. During the Second World War, Brian, Hemming and McGowan investigated this at ICI’s Jealott Hill Research Station in Bracknell.\(^5\) They discovered that the common soil microbes were largely absent from Wareham Heath soil and in their place was an abundance of *Penicillia*. When cultures of these were screened against a fungus known as *Botrytis allii*, a strain of *Penicillium janczewskii* produced a peculiar distortion of the germ tubes of the fungus, even at high dilutions. An active substance was extracted with chloroform and crystallised from alcohol, this being named ‘curling factor’ until identified as griseofulvin,\(^5\) a mould metabolite previously isolated from *Penicillium griseofulvum* by Harold Raistrick, though not screened for antibiotic activity.\(^5\)

The chemical structure of griseofulvin was elucidated in 1952 by John Grove and his colleagues at the Akers Research Laboratories of ICI.\(^5\) Its stereochemistry was established later\(^5\) and the first synthesis was reported in 1960.\(^6\)
ICI had intended to introduce griseofulvin for eradication of fungal diseases in plants, but its early promise was never fulfilled. Matters might have rested there had not a group of Polish scientists at the Municipal Hospital in Poznan published a report in November 1957 describing their spectacular success in treating fungal infections with salicylhydroxamic acid.\textsuperscript{57} Their clinical studies broke new ground, for they took the unprecedented step of administering the compound by mouth. This was noted by James Gentles, a mycologist in the Department of Bacteriology at Anderson’s College in the University of Glasgow, who had been seeking a remedy for fungal infection of the feet of coal miners. He administered griseofulvin by mouth to guinea pigs that had developed severe lesions after having been infected with \textit{Microsporum canis}. The beneficial effects of the antibiotic were evident within four days.\textsuperscript{58} Further studies revealed that griseofulvin could prevent and cure ringworm in cattle. Furthermore, when griseofulvin was administered by mouth over a prolonged period of time, it selectively concentrated in keratin and so was of value in the treatment of dermatophyte infections of skin or nails. Griseofulvin then became the first orally administered antifungal drug to be marketed when ICI and Glaxo introduced it in 1959, the latter having developed a fermentation plant that permitted economical production.

\textbf{Ciclosporin}

The Sandoz Company in Basle began testing fungal metabolites for cytostatic activity in 1957, employing two screening tests introduced by Hartmann Stähelin. These involved detection of the inhibition of growth of either murine P-815 mastocyтомa cells or chick embryo fibroblasts. Thousands of filtered fungal cultures were examined, leading to several active compounds being isolated, including the cytochalasins, brefeldin A, verrucarin A, anguidine and chlamydocin.\textsuperscript{59} Like many other fungal metabolites obtained by pharmaceutical companies, none of these ever found a place in medicine. However, in 1965 the sesquiterpene ovalicin was isolated from the culture fluid of \textit{Pseudeurotium ovalis} and shown to have potent cytostatic activity. Shortly after this, Sandoz widened its interests to include immunology. Sandor Lazary then set up a screening programme to find an immunosuppressant drug with less toxicity than azathioprine. As ovalicin had reduced the weight of the spleen in mice, Stähelin asked for it to be tested in the mouse haemagglutinin test, which reflected the degree of antibody production. Ovalicin proved to be a potent immunosuppressant in this and a variety of other tests in animals. Significantly, it was not toxic to the cells of the bone marrow, which distinguished it from existing immunosuppressants. Unfortunately, when tested in humans it turned out to be too toxic and had to be abandoned.

Sandoz introduced a general screening programme in 1970, through which products prepared in its various laboratories could be submitted to a battery of screens for a broad range of activity. Screening for immunosuppressive activity was included, involving a novel procedure developed by Stähelin in which both activity against leukaemia L1210 cells and also a haemagglutination assay could be conducted in the same mouse. The latter involved injecting a mouse with red blood cells from sheep to produce an immune response. Compounds being screened for immunosuppressant activity were then injected intraperitoneally for four days. Nine days later, blood was taken from the mouse and the level of antibodies in the serum was measured.
Among 20 compounds submitted to Stähelin for screening in December 1971 was an extract from *Tolypocladium inflatum*, a new strain of fungi imperfecti that had been active when tested for antifungal activity. It was then produced in greater amounts and an extract was partially purified by Arthur Rüegger and his colleagues. This product was not active *in vivo* against pathogenic fungi, but when it was screened for immunosuppressive activity in the mouse the results were quite different. The antibody count in the haemagglutinin test was reduced by a factor of 1024, indicating a strong immunosuppressant effect, while there was no activity in the L1210 test. Further tests on P-815 mastocytoma cells were also negative. This placed the product in a unique category. All known immunosuppressant drugs had achieved their effects by a non-specific suppression of mitosis, not just in lymphocytes but in all rapidly dividing somatic cells. The indication now was that this product acted selectively on lymphocytes involved in the immune response.

The fermentation process was now improved and the individual components of the product were separated by column chromatography, resulting in the isolation of the pure peptides cyclosporin A and B and the determination of their structures. Since most of the immunosuppressant activity was associated with cyclosporin A, only it was used in further researches. It now has the approved name of ‘ciclosporin’.

The first report revealing the work that had been carried out at the Sandoz laboratories appeared in 1976. Samples of the drug were then supplied to Roy Calne (who had been closely involved in the development of azathioprine) for clinical trial in patients receiving kidney transplants at Addenbrooks Hospital in Cambridge, and also to Ray Powles at the Leukaemia Unit in the Royal Marsden Hospital, London, where its role in bone marrow transplantation was to be assessed. Their findings appeared in 1978. Since then, the value of ciclosporin in dealing with the commonest cause of death after transplantation, namely rejection, has been confirmed by its successful use in hundreds of thousands of patients. It has to be given for at least six months after transplantation, the main side effects involving the kidneys.

**ACTINOMYCETAL ANTIBIOTICS**

The actinomycetes are ubiquitous soil organisms that have features in common with both bacteria and fungi. In 1917, Greig-Smith detected antibiotic substances diffusing from actinomycetes soil samples gathered in New South Wales. Rudolf Lieske then reported that while some actinomycetes lysed the cells of *Staphylococcus aureus* and *S. pyogenes*, they had no effect on other bacteria. At first unaware of the prior work of Lieske, André Gratia and his group at the Pasteur Institute in Brussels began publishing a series of papers in which substances diffusing from various species of *Streptothrix* and *Actinomyces* were employed to lyse bacteria in culture in order to liberate antigens. The resulting ‘mucolysates’ were administered to patients in attempts to induce formation of antibodies that would produce immunity against the specific
bacterium from which they had been prepared.68 These experiments were not particularly successful. Maurice Welsch at the Rockefeller Institute later gave the name ‘actinomycetin’ to the soluble protein-like mucolysate.69

**Streptomycin and Other Aminoglycoside Antibiotics**

During his work leading to the isolation of tyrothricin, Rene Dubos had kept in contact with Selman Waksman, his former teacher in the Department of Microbiology at the New Jersey Agricultural Experiment Station in Rutgers University. As a result, Waksman became convinced that his own wide experience in dealing with soil microbes could be used to good effect in seeking further antibiotic-producing organisms. He chose to begin with actinomycetes, he and his students starting with a preliminary survey of these organisms. This confirmed findings of earlier investigators by establishing that, out of 244 freshly isolated cultures from soil, over 100 had antimicrobial activity, of which 49 were highly active. Waksman then used the pyocyanase isolation procedure to obtain from *Actinomyces antibioticus* a crystalline antibiotic known as actinomycin A, the first antibiotic ever isolated from an actinomycete.70 After routine bacteriological tests, it was sent to the nearby laboratories of Merck and Company in Rahway for further investigation. Though considerable effort was spent in attempting to elucidate its chemical structure, and many animals were used to assess its toxicity and potential clinical scope, it proved too toxic for human application.

Waksman was now awarded an annual grant of $9600 from the Commonwealth Fund to support his search for antibiotics. Further funds were forthcoming from Mrs Albert Lasker. Waksman, like Florey and Ehrlich before him, thus received his main financial support for his chemotherapeutic research from charitable foundations. The similarity goes further, for all three subsequently witnessed their discoveries being rapidly brought to the clinic as a consequence of the readiness of major pharmaceutical firms to commit large sums of money in the exploitation of these discoveries.

In 1941, Waksman isolated two more antibiotics, clavacin (patulin) and fumigatin.71 They were less toxic than actinomycin A, but still unsuitable for clinical application. When it became evident that penicillin was active only against Gram-positive bacteria, Waksman concentrated on the search for an antibiotic to treat Gram-negative infections. He appeared to have achieved a breakthrough when he isolated streptothricin, as it was lethal to bacteria unaffected by penicillin and appeared to be safe enough for human trials. Several industrial concerns were given cultures of the actinomycete that produced it, and pilot plant production was begun. Unfortunately, chronic toxicity testing revealed that several days after streptothricin was injected into animals a variety of toxic effects appeared as a result of kidney damage. Work on it had to be abandoned.

Early in 1943, Waksman decided to focus his activities on finding an antibiotic that could be used to treat tuberculosis, a major scourge that caused millions of deaths each year. The problem he faced was that the causative organism, *Mycobacterium tuberculosis*, grew very slowly, making the screening of cultures impracticable on the scale Waksman was operating on. Acting on a suggestion from his son, he instead screened his cultures against the faster growing *Mycobacterium phlei*, a non-pathogenic organism. Any cultures that were active were then examined further in animals infected with tuberculosis. Waksman also enriched his numerous soil samples with *Mycobacterium tuberculosis* in order to encourage elaboration of actinomycetes that produced an antituberculosis antibiotic.

After having cultured thousands of strains of actinomycetes since the start of the project, Waksman found what he wanted in September 1943. By a twist of fate, this was an actinomycete that he and his former professor had been the first to isolate 28 years earlier at Rutgers, namely *Streptomyces griseus*. Within four months, a new antibiotic had been isolated in a concentrated form, and its activity against *Mycobacterium tuberculosis* confirmed. It also proved effective
against microbes causing plague, brucellosis and various forms of bacterial dysentery. Although its chemical properties turned out to be similar to those of streptothricin, the new antibiotic was free of renal toxicity. It was given the name ‘streptomycin’. The chemical structure was elucidated in 1947 and confirmed its aminoglycoside nature. It was later established that streptomycin and other aminoglycoside antibiotics disrupt translation from messenger RNA (mRNA) to protein in bacterial ribosomes, thereby blocking protein biosynthesis.

After initial tests on guinea pigs infected with tuberculosis, the first clinical trials with streptomycin on patients began. When the early results proved encouraging, it was agreed that the US National Research Council should co-ordinate large-scale trials in order to hasten progress, such was the pressing need for a cure for tuberculosis. The cost of the co-ordinated programme was met entirely by the pharmaceutical industry. By the time the trials were completed and large-scale production of streptomycin had begun, the US industry alone had spent 20 times as much again on production plant.

For many years streptomycin was used as a first-line drug for the treatment of tuberculosis, but large doses were found to damage the aural nerve, causing deafness. Fortunately, the discovery of synthetic antituberculosis drugs eased this problem by permitting them to be used in combination with smaller doses of streptomycin.
Waksman isolated neomycin from *Streptomyces fradiae* in 1949.\(^7^5\) It was an antibiotic complex, the component used therapeutically being neomycin B. The structure of neomycin B was fully elucidated in 1962\(^7^6\) and it was synthesised in 1987.\(^7^7\) Framycetin, an antibiotic isolated by French researchers in 1954, was shown to be identical to neomycin B.\(^7^8\) Because of its neurotoxicity, neomycin is restricted to topical use for skin, ear and eye infections.

Kanamycin is an antibiotic complex isolated in 1957 by Hamao Umezawa at the Institute of Microbial Chemistry in Tokyo, where he had been running an antitumour screening programme utilising the transplanted Yoshida sarcoma in mice.\(^7^9\) The structure of the major component, kanamycin A, was established in 1958\(^8^0\) and it was synthesised ten years later.\(^8^1\) It is another toxic aminoglycoside antibiotic which is reserved for the treatment of penicillin-resistant staphylococcal and serious Gram-negative infections resistant to gentamicin.

Lincomycin was isolated in 1962 from *Steptomyces lincolnensis* in a soil sample gathered in Lincoln, Nebraska.\(^8^2\) Its structure was determined two years later and it was synthesised in 1970.\(^8^3\) It was a highly toxic drug and has been superseded by its derivative clindamycin, which has similar activity but is absorbed from the gut more efficiently.

Gentamicin C\(_1\) was isolated from a complex of antibiotics produced by *Micromonospora purpurea* and *M. echinospora* by researchers at the Schering Corporation in Bloomfield, New Jersey, in 1963.\(^8^4\) Its structure was determined in 1967.\(^8^5\) Gentamicin became the aminoglycoside of choice for a variety of purposes, being active against pseudomonal infections, unlike kanamycin. However, resistance gradually developed and has limited its value.

Several other aminoglycosides have been introduced into medicine, including amikacin, netilmicin and tobramycin.

**Chloramphenicol**

Yale botanist Paul Burckholder received a grant of $5000 from Parke, Davis and Company in 1943 to screen soil samples for antibiotic activity against six selected types of bacteria. He examined more than 7000 samples from all over the world, including one collected by his
friend Derald Langham, an agricultural geneticist who was carrying out research near Caracas in Venezuela. An unknown antibiotic-producing actinomycete was isolated from this sample and given the name *Streptomyces venezuelae*. A culture was sent to Parke, Davis and Company in Detroit, where John Ehrlich and Quentin Bartz\textsuperscript{86,87} isolated chloramphenicol in 1947. It turned out to be an orally active, broad-spectrum antibiotic.

The first patients to receive chloramphenicol were victims of an epidemic of typhus sweeping through Bolivia. In December 1947, 22 patients in the General Hospital at La Paz, of whom at least five were close to death, were given injections of the new antibiotic by Eugene Payne, a clinical researcher from Parke, Davis and Company. All were cured. Similar results were obtained in Kuala Lumpur, but this time there was some confusion that resulted in patients with typhoid also receiving injections of chloramphenicol. Their rapid recovery was unprecedented.

Parke, Davis and Company researchers rapidly elucidated the chemical structure of chloramphenicol and then synthesised it.\textsuperscript{88} By 1949, large amounts of chloramphenicol were being manufactured, with sales that year exceeding $9 million. They increased fivefold over the next two years, turning Parke, Davis and Company into the largest pharmaceutical company in the world. Some eight million patients were treated with this apparently safe antibiotic before reports that patients had died from aplastic anaemia caused by it appeared in leading medical journals. The incidence has been estimated as between 1 in 20 000 and 1 in 100 000, with 80\% of the victims dying. Had the drug not been rushed on to the market, the number of cases would hardly have caught the public eye. However, the *Journal of the American Medical Association* published a warning against the promiscuous use of chloramphenicol and then issued a press release that was widely reported. The available evidence was studied by the Food and Drug Administration, with the result that Parke, Davis and Company were permitted to continue selling the drug, but with a warning on the package. This stated that prolonged or intermittent use could cause blood disorders. Although sales dropped markedly for a year or two, the drug regained some of its popularity when the problem of resistant strains of staphylococci became more common, but the arrival of the broad-spectrum penicillins largely replaced it for many purposes. Today, chloramphenicol is reserved for the treatment of typhoid, salmonella, meningitis and rickettsial infections. It is also used topically in treating eye and ear infections. No superior analogue ever emerged, despite the synthesis of many compounds. It acts by binding exclusively to the 50S subunit of bacterial ribosomes, inhibiting the enzyme peptidyl transferase. This prevents peptide bond formation.

**The Tetracycline Antibiotics**

Benjamin Duggar, a 71 year old retired botany professor from the University of Wisconsin, became a consultant to Lederle Laboratories at Pearl River, New York, in 1943 to investigate a plant alleged to have antimalarial activity. A year later he was asked to supervise the screening of hundreds of soil samples in order to find a safer antibiotic than streptomycin for treatment of tuberculosis. In the summer of 1945, a sample was received from William Albrecht of the University of Missouri, where Duggar had been a member of faculty 40 years earlier. This contained a golden actinomycete with antibiotic activity. Duggar named it *Streptomyces aureofaciens*.\textsuperscript{89} The antibiotic was isolated and later given the approved name of
‘chlortetracycline’ after its chemical structure was elucidated. Tests showed it to be an orally active, broad-spectrum antibiotic with a therapeutic profile similar to that of chloramphenicol, but with no value in tuberculosis. By December 1948, large-scale production in fermentation tanks had begun. The process was patented and the antibiotic was put on the market shortly before chloramphenicol.90

Robert Woodward at Harvard and chemists from the Pfizer laboratories in Brooklyn established the chemical structure of chlortetracycline in 1952 at the same time as that of oxytetracycline, in what is generally considered to be a classic example of structural elucidation.91 The first total chemical synthesis of the tetracyclines was reported in 1959 by Lederle chemists.92

Like other tetracyclines, chlortetracycline inhibits protein synthesis by binding to the 30S subunit of bacterial ribosomes and interfering with the binding of aminoacyl transfer RNA (tRNA). The selectivity for bacteria arises from a transport process that occurs in both Gram-positive and negative bacteria, but tetracyclines also diffuse by passive transport into bacterial cells and, if the concentration is sufficiently high, into mammalian cells, where protein synthesis can also be impaired. However, chlortetracycline has poor oral bioavailability and a shorter half-life than other tetracyclines, hence it is less appropriate than those for the treatment of systemic infections.

The introduction of chlortetracycline and chloramphenicol threatened the market position of Pfizer as the leading producer of penicillin, the price of which was already plummeting. In response, the company set up a team of 11 researchers and 45 assistants who went on within the next 18 months to examine almost 100 thousand soil samples from around the world. The antibiotic that emerged was cultured from a soil sample containing Streptomyces rimosus, which was collected near Pfizer’s Terre Haute factory! This was followed by the rapid isolation of oxytetracycline.93 It was quickly purified and found to have similar properties to chlortetracycline.94 A patent was applied for at the end of November 1949.95

Pfizer decided to market oxytetracycline themselves, rather than supply it in bulk to others as it had done with all its previous products. Lacking a sales force of medical representatives who could call on physicians, they indulged in what proved to be a much criticised campaign of direct advertising to the general public. The cost of developing oxytetracycline had been about $4 million, but in two years the company spent almost double this amount on advertising. This resulted in their obtaining a quarter of the American market for broad-spectrum antibiotics in 1951, roughly the same share as chloramphenicol.

In 1952, Lederle researchers isolated tetracycline from the actinomycete they were using to produce chlortetracycline,96 and Bristol Laboratories obtained it from Streptomyces
viridifaciens. Around the same time, Lloyd Conover of Pfizer obtained tetracycline when the chlorine atom of chlortetracycline was removed by catalytic hydrogenation. These three American companies all applied for patents in 1952–1953, with Lederle and Pfizer agreeing to cross-license each other. All the applications were rejected, but Pfizer and Bristol fought the decision and succeeded in obtaining patents in 1955. A subsequent agreement made between the tetracycline producers and their licensees was criticised by the US Federal Trade Commission in 1958, on the grounds that it was an attempt to eliminate competition and fix prices. This was denied by the companies.

The three original tetracyclines were sought after eagerly when first introduced. They acquired a popular reputation as miracle drugs, vying with penicillin. Their commercial success was due to their oral activity, the first penicillin suitable for oral administration not being introduced until the mid-1950s. However, the proportion of these early tetracyclines absorbed from the intestine was only a fraction of the total dose, leading to disturbance of the normal bacterial flora of the gut. In hospitalised patients, this gradually led to replacement of the flora by strains of resistant bacteria, leading to a decline in their use in hospital practice. Nevertheless, some tetracyclines remain the drugs of choice in trachoma, psitacosis and similar chlamydial infections, as well as in those caused by rickettsia, mycoplasma and brucella. They are also effective against *Haemophilus influenzae* and so may be of value in chronic bronchitis.

![Demeclocycline](image)

In 1957, demeclocycline was isolated from mutant strains of *Streptomyces aureofaciens* by Lederle researchers. It had improved chemical stability due to the absence of an unstable tertiary alcohol function in the tetracycline ring system, the alcohol function now being secondary. This resulted in enhanced blood levels and encouraged chemists to develop semi-synthetic tetracyclines in which there was no tertiary alcohol function.

### Macrolide Antibiotics

Robert Bunch and James McGuire of Eli Lilly and Company isolated erythromycin in 1952 from a strain of *Streptomyces erythreus* cultured from a soil sample collected in Iloilo in the Philippines. The chemical structure was determined in 1956. It was the first of the macrolide antibiotics (so named because of the presence of a large ring in their chemical structure) to be introduced into the clinic. It binds to the 50S subunit of bacterial ribosomes, inhibiting the translocation stage of protein synthesis. This type of action makes it a bacteriostatic rather than a bacteriocidal antibiotic.

![Erythromycin](image)
Although the chemical structure of erythromycin bears no relation to penicillin, its spectrum of action is similar. This renders it of value in patients allergic to penicillin and in the treatment of penicillin-resistant staphylococcal infections. The principal use of erythromycin is in the treatment of Gram-positive infections of the skin, soft tissues and respiratory tract.

Erythromycin is unstable in gastric juice, so oral administration leads to erratic plasma levels. Furthermore, the decomposition products are responsible for a high incidence of nausea and epigastric pain. Several esters and salts of erythromycin that are insoluble in gastric juice have been introduced to avoid gastric acid decomposition. Also, by formulating erythromycin base in enteric-coated tablets, degradation is largely prevented.

A novel macrolide was discovered by Surendra Sehgal and his colleagues at the Ayerst laboratories in Montreal when they isolated an antifungal antibiotic from *Streptomyces hygroscopicus* in 1972. They named it ‘rapamycin’ because the streptomycete had been found in a soil sample collected seven years earlier by the Canadian Medical Expedition to Easter Island, the local name of which was Rapa Nui. The structure was determined in 1978 and it was synthesised some years later.

Rapamycin was found to be active against *Candida albicans* and some other fungi, but when detailed studies were conducted it was found that it had a powerful suppressant effect on the immune system. This resulted in rapamycin being dropped as a potential antifungal, but then it was investigated as an immunosuppressant drug. In addition, tests conducted by the National Cancer Institute revealed that it possessed promising activity as a cytostatic drug when used in combination with cytotoxic agents. With such
exciting prospects for rapamycin, the sudden closure of the Montreal research facility and
termination of all projects must have been a terrible blow for the staff concerned. Fortunately,
Sehgal had the foresight to harvest a final batch of the antibiotic from the fermentation tanks
and store it safely when he was transferred to the Princeton laboratories of the company.
However, it was not until Ayerst was merged with Wyeth in 1987 and new management was in
place that he was able to persuade anyone to reinstate the project. Fortunately, steady progress
was then made and rapamycin was licensed by the Federal Drug Authority (FDA) in 1999 for use
in kidney transplantation. It now has the international non-proprietary name (INN) of sirolimus
and is being clinically investigated for its potential in various types of cancer.

Researchers at the Fujisawa Pharmaceutical Company in Osaka, Japan, isolated an
antibiotic from *Streptomyces tsukubaensis* in a soil sample taken from the foot of Mt Tsukuba,
near Tokyo, in 1984.104 The structure was elucidated in 1987,105 with a total synthesis being
achieved within two years by Ichiro Shinkai and his colleagues at Merck.106

After it was found to inhibit T-cell activation, tacrolimus was investigated as an
immunosuppressant.107 Positive results were followed by clinical trials at the University of
Pittsburg in patients whose liver transplants were being rejected after suppressant therapy.
Within ten years of its isolation, tacrolimus was licensed for use in liver and kidney transplant
patients. It is more toxic than ciclosporin.

Another macrolide antibiotic was discovered in 1977 as the major component in a mixture
of avermectins isolated from *Streptomyces avermitilis* by Merck researchers. The components
were separated and then their structures were elucidated.108 Abamectin was selected as it had
anthelmintic activity, but it is only used in animals.109 Its chemical reduction product,
ivermectin, will be discussed in the next chapter.

Rifamycin Antibiotics

Following their entry into the field of penicillin production, Lepetit Research Laboratories of
Milan established a screening programme to discover new antibiotics. This led to the isolation
from a soil sample collected on the Cote d’Azur in the summer of 1957 of a strain of
*Streptomyces* that, when cultured, exhibited high activity against Gram-positive bacteria and
*Mycobacterium tuberculosis*.110 The micro-organism was initially classified as *Streptomyces
mediterranei*, but later evidence led to its reclassification as *Nocardia mediterranei*.

The available chemical evidence from investigation of a brown powder obtained from the
fermentation broth indicated that its activity was due to a group of at least five novel
antibiotics. These were given the generic name of ‘rifamycins’, this term being chosen from the
title of a Jules Dassin gangster film, namely ‘Rififi’ (French argot: *rififi* = a struggle).111 Due to
stability problems, the only crystalline antibiotic isolated from the original mixture was
rifamycin B. It constituted less than 10% of the total antibiotic titre. However, another strain of *N. mediterranei* was found to produce high yields of this.

Rifamycin B had moderate antibiotic activity when administered to animals, but this was not sufficient to justify it being considered for potential therapeutic application. However, aqueous solutions of the antibiotic acquired stronger antibacterial activity on standing. Investigation of this phenomenon led to the clinical introduction of rifamycin SV as it was active against a wide range of organisms, including *Mycobacterium tuberculosis*, *M. leprae*, Gram-positive bacteria and some Gram-negative bacteria.

Victor Prelog and Wolfgang Oppolzer of the Swiss Federal Institute of Technology (ETH) in Zurich determined the chemical structure of rifamycin B in 1964.

**Polyene Antibiotics**

Nystatin, an antifungal polyene antibiotic produced by various species of *Streptomyces* including *S. noursei* and *S. aureus*, was introduced by Squibb after its discovery in 1950 by Elizabeth Hazen and Rachel Brown during an extensive soil survey conducted by the New York State Department of Health. The correct structure was established in 1976. As nystatin is too toxic for systemic treatment, its use has been limited to either topical application for *Candida albicans* infection of the skin or, as it is not absorbed from the gut, oral administration to treat intestinal candidiasis.
Amphotericin was isolated in 1953 from a strain of *Streptomyces nodosus* growing in a sample of Orinoco river soil that had been sent from Venezuela to the Squibb Institute for Medical Research.\textsuperscript{115} The complete structure was confirmed in 1976.\textsuperscript{114} Amphotericin is the only polyene antibiotic safe enough to be administered parenterally, being used to combat life-threatening systemic fungal infections in patients whose immune system is compromised as a consequence of intensive cancer chemotherapy or AIDS. It is structurally related to nystatin.\textsuperscript{116} It enters the fungal cell membrane, where it disrupts membrane integrity by binding to ergosterol. This causes loss of ions which are critical for the normal functioning of the fungal cell. Unfortunately, amphotericin can also damage human cell membranes, making it one of the most toxic drugs in current clinical use when administered parenterally.

**Azomycin**

Nakamura and Umezawa isolated azomycin in 1953 from an unidentified species of *Streptomyces*.\textsuperscript{117} Its structure was determined by Nakamura\textsuperscript{118} and it was synthesised in 1965.\textsuperscript{119}

\[
\text{O}_2\text{N}\begin{array}{c}
\text{N}
\end{array}\begin{array}{c}
\text{H}
\end{array}
\]

azomycin

As azomycin was structurally similar to the trichomonicidal agents aminitrozole and 2-amino-5-nitrothiazole, Rhône Poulenc researchers tested it and found it to have similar activity, but it turned out to be too toxic for clinical exploitation. Analogues were then prepared and metronidazole was found to be not only an antiprotozoal drug but also an antibacterial one. This is discussed in the next chapter.

**Vancomycin and Teicoplanin**

Vancomycin was isolated from *Streptomyces orientalis* at the Lilly laboratories in 1955,\textsuperscript{120} but its structure was not correctly assigned until 1981.\textsuperscript{121} Vancomycin was effective against both aerobic and non-aerobic Gram-positive bacteria, especially resistant staphylococci, acting by disrupting bacterial cell wall synthesis. Early preparations were contaminated with impurities that caused a high incidence of side effects. As a result of its purification, vancomycin became the drug of choice for the oral treatment of pseudomembranous colitis since it was not absorbed from the gut.
Teicoplanin is a complex of five antibiotics isolated from *Actinoplanes teichomycetius* by Lepetit researchers in 1976. The components were separated in 1983 and then identified. The long side chain attached to one of the sugar rings varied in each of the five components, teicoplanin A2-3 being shown here. Teicoplanin has similar activity to vancomycin, but has a longer half-life and hence can be administered once daily. As it is less irritant than vancomycin, it can be injected intramuscularly or intravenously.

**Thienamycin**

The carbapenems are a group of exceedingly potent antibiotics isolated from streptomycetes, more than 40 having been reported by 1990. Their potency may be due to superior penetration into bacterial cells. The most effective of them is thienamycin, which was isolated in 1976 from *Streptomyces cattleya* by Merck investigators in the course of screening for inhibitors of peptidoglycan synthesis. Its structure was determined two years later. Early indications were that thienamycin was resistant to beta-lactamases and was as active as gentamicin against Gram-negative organisms. However, it had poor activity when administered by mouth and was chemically unstable. Consequently, it is only used in the form of a stable derivative known as imipenem (discussed in the next chapter).

**Clavulanic Acid**

Concern about the growing problem of resistance to penicillins and cephalosporins led Rolinson of Beecham Pharmaceuticals to consider the possibility that beta-lactamase inhibitors might be produced by micro-organisms. A screen was set up to spot fungal or actinomycete extracts that could restore the sensitivity towards benzylpenicillin of a beta-lactamase-producing strain of *Klebsiella aerogenes*. If a beta-lactamase inhibitor was present in an extract, no growth of *K. aerogenes* would occur. When isolates of *Streptomyces clavuligerus* exhibited high beta-lactamase inhibitory activity, clavulanic acid was extracted. Although it had no significant antibacterial activity, it was a potent inhibitor of beta-lactamases from a variety of bacterial species. When combined with amoxycillin, it overcame resistance from organisms that
produced β-lactamases. This combination proved satisfactory in clinical trials and is still prescribed for treatment of respiratory or urinary tract infections. Combinations of clavulanic acid and other antibiotics have been marketed.

**CYTOTOXIC ANTIBIOTICS FROM STREPTOMYCETES**

After Selman Waksman had isolated actinomycin A, Hans Brockmann at Göttingen University obtained a second actinomycin antibiotic from *Streptomyces chrysomallus* in 1949, which he named actinomycin C. Later, this was shown to be a complex consisting of three components, named actinomycin CD₁, CD₂ and CD₃. Following a report from the Sloane–Kettering Institute that Waksman’s actinomycin A had detectable activity against sarcoma-180, Brockmann sent his crude actinomycin C to the Bayer Institute for Experimental Pathology at Elberfeld, where it was found to inhibit tumour growth. It was subsequently found to be effective in some patients with lymphatic tumours at dose levels that did not produce toxic effects, though this was disputed. Nevertheless, actinomycin C was marketed for some years, principally for use in Hodgkin’s disease, until rendered obsolete by more effective agents.

In 1953, Waksman isolated actinomycin D from *Streptomyces parvullus*, the first organism to yield a single actinomycin rather than a complex mixture. It turned out that this was identical to actinomycin CD₁, and both of these terms were later replaced by the approved name of ‘dactinomycin’. Pure dactinomycin, which constituted about 10% of the actinomycin C complex, was superior to actinomycins CD₂ and CD₃ as an antitumour agent. Its chemical structure was established in 1957 and it was synthesised by Brockmann in 1964. The other actinomycins have similar structures, differing only in two of their amino acids.

Dactinomycin showed marked antitumour activity against many transplanted mouse tumours, occasionally causing total regression of some of them. As soon as toxicological studies were completed, a clinical trial was initiated in children with acute leukaemia. Disappointingly, dactinomycin was ineffective. Nevertheless, encouraging results were achieved in children with advanced Wilm’s tumour, rhabdomyosarcomas (muscle tumours), Ewing’s tumour of the bone and Hodgkin’s disease. The prospects of survival for children with Wilm’s tumour of the kidney were completely transformed when a combination of radiotherapy and sustained chemotherapy with dactinomycin on its own or with vincristine was introduced. When maintained for up to two years, 90% of children are cured, which is three times as many as was the case prior to the introduction of dactinomycin. The main value of it in adults appears to be for the treatment of soft tissue sarcomas and testicular teratomas.
Bleomycin

In 1962, Umezawa detected antitumour activity while screening culture filtrates of *Streptomyces verticillus*. He managed to isolate the bleomycins from this, a complex group of glycopeptides of which bleomycin A₂ is the main component of the product used clinically.\(^{136,137}\)

![Bleomycin structure](image)

The most striking feature of bleomycin is the fact that it is one of the few anticancer drugs that does not cause bone marrow depression. However, it may cumulatively damage the lung, especially in elderly patients. Bleomycin is administered parenterally in the treatment of squamous cell carcinoma (cancer in flattened lining cells), head and neck tumours, non-Hodgkin’s lymphoma and testicular teratomas.

**Anthracycline Antibiotics**

Daunorubicin (daunomycin, rubidomycin) was isolated in 1962 from *Streptomyces peucetius* by Di Marco and his colleagues of the Farmitalia Company in Milan.\(^{138}\) The chemical structure was established two years later.\(^{139}\) This particular streptomyecete had been investigated after extracts of a soil sample from Apulia (where the Peucetii and Daunii tribes had lived in ancient times) yielded material with activity against cultures of murine Ehrlich carcinoma. At the Instituto Nazionaleper lo Studio e le Cura dei Tumori in Milan, where Di Marco had previously worked, daunorubicin was shown to exhibit actinomycin-like activity against tumours, intercalating with DNA to prevent it from serving as a template for replication.

![Daunorubicin and Doxorubicin structures](image)
When administered to animals with tumours, daunorubicin was more active than actinomycin C. Its clinical value was limited by severe toxicity to the heart, but it found some use in the combination chemotherapy of leukaemia, particularly in helping to induce remissions in cases where the patient had failed to respond to safer drugs.

Another antibiotic isolated by Farmitalia scientists from *S. peucetius* in 1967 was named ‘doxorubicin’, which turned out to be the 14-hydroxy derivative of daunorubicin. This apparently minor chemical difference transformed it into one of the most successful antitumour drugs ever discovered, but the problem of cardiotoxicity remained. Patients who received a cumulative dose in excess of 550 mg/m² were at risk of having a heart attack induced by the affinity of the drug for cardiac muscle. Nevertheless, when doxorubicin is administered with care it is of considerable value in the treatment of acute leukaemias, lymphomas (non-Hodgkin’s) and many solid tumours. Aclarubicin, which has similar uses, was one of 20 aclacinomycins isolated from *Streptomyces galilaeus* by Umezawa in 1974.

**MONOBACTAM ANTIBIOTICS**

Selective screening techniques applied to soil micro-organisms resulted in the isolation of sulfazecin and other novel monobactams from the bacteria *Pseudomonas acidophila* and *P. mesoacidophila* by Akira Imada at the Takeda laboratories in Osaka and by Richard Sykes and his colleagues at the Squibb Institute for Medical Research. Sykes also isolated SQ 26180.

Although these natural antibiotics were active against Gram-negative bacteria, they were not considered to be effective enough for clinical application.

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Antibiotic Analogue

Just as with plant products and biochemical substances from mammalian sources, analogues of naturally occurring antibiotics were prepared in order to overcome disadvantages that had become manifest. The earliest of these were analogues of benzylpenicillin.

6-AMINOPENICILLANIC ACID

John Sheehan, one of the leading Merck chemists working on the synthesis of penicillin, continued his work in this area after he left the company to become Professor of Chemistry at Massachusetts Institute of Technology. With his research financially supported by Bristol Laboratories, Sheehan devised novel techniques that would make it possible to synthesise the unstable beta-lactam ring, the stumbling block that had thwarted all previous attempts. In 1957, he finally synthesised phenoxymethylpenicillin. The overall yield was around 1%, but within two years Sheehan had increased this to more than 60%. His synthesis enabled him to prepare 6-aminopenicillanic acid, the key to making analogues with novel side chains by reacting it with acid chlorides. This was a major improvement on the only alternative method of obtaining new penicillins, namely through addition of a chemical precursor to the liquor in which the Penicillium mould grew.

Sheehan’s synthesis of 6-aminopenicillanic acid would have been difficult to scale-up and develop for commercial application, but the problem was avoided by a separate development the following year. Ralph Batchelor, Peter Doyle, John Nayler and Rolinson of the recently established Beecham Research Laboratories at Betchworth, Surrey, made a remarkable discovery. They were newcomers to the field of penicillin research and had been advised by Ernst Chain to prepare 4-aminobenzylpenicillin as it might be possible to make novel derivatives from this. In the course of extracting the new penicillin, its acetyl derivative crystallised out of solution as a pure compound. Since the crystals could easily be converted to the desired 4-aminobenzylpenicillin, it was obvious that addition of an acetylating agent to the mould juice would facilitate the isolation process by converting all of the 4-amino compound to the less soluble acetylamino derivative. When this was done, a discrepancy appeared in the microbiological assay of the mould juice, which now indicated enhanced antibacterial activity. In an inspired interpretation of this, the Beecham researchers concluded that 6-aminopenicillanic acid must have been present in the mould juice. Only after conversion to its acetyl derivative did it exhibit sufficient activity to affect the assay result. Subsequent tests confirmed that 6-aminopenicillanic acid was always present in mould juice, and was a stable substance, contrary to expectation. The Beecham team quickly exploited their discovery...
by developing methods of obtaining large quantities of this key intermediate by fermentation and their first patent was applied for in August 1958.

The availability of large amounts of 6-aminopenicillanic acid was a turning point in antibiotic chemotherapy, for it was now easier to make novel semi-synthetic penicillins rather than indulging in expensive screening of soil samples in the hope of finding that rare product – a non-toxic antibiotic. Most of the new antibiotics introduced since the early 1960s have been intended for the treatment of conditions that would not be expected to respond to penicillin therapy, such as non-bacterial infections and cancer.

Since Beecham Research Laboratories employed Sheehan’s method of converting 6-aminopenicillanic acid into therapeutically useful penicillins, meetings were held with him and representatives of Bristol Laboratories. Early agreement was reached whereby both companies would collaborate in the development of semi-synthetic penicillins. After this, harmony between the three parties rapidly dissipated. Bristol and Beecham went their own ways, and for the next 20 years there were interminable legal wrangles between Sheehan and Beecham over patent rights to the new semi-synthetic penicillins. The matter is too complex to be dealt with here, but suffice it to say that the US Board of Patent Interferences ruled in favour of Sheehan in 1979. He has written his own account of what transpired.3

Phenoxymethylpenicillin

The ability to synthesise new penicillins at will permitted the shortcomings of benzylpenicillin (penicillin G) to be tackled. It had been the only penicillin in general use until the mid-1950s, when phenoxymethylpenicillin (penicillin V) was introduced. This was one of a number of penicillins originally obtained in 1948 by scientists at the Lilly Research Laboratories after they had pioneered the technique of adding different chemical precursors to the culture medium in which the *Penicillium* mould was fermented. Its true value was not recognised until four years later when Ernst Brandl, a chemist in an Austrian penicillin production plant who was attempting to extract phenoxymethylpenicillin, noticed that it had the unique property of being resistant to degradation by dilute acid.4 This meant that it should withstand exposure to gastric acid, thus avoiding the extensive degradation when benzylpenicillin was taken by mouth. Eli Lilly and Company exploited this by introducing the potassium salt of phenoxymethylpenicillin as the first reliable orally active penicillin. It is not used to treat serious infections as it is less active than benzylpenicillin and plasma levels vary after oral administration. It is prescribed mainly for respiratory infections in children.

The acid stability of phenoxymethylpenicillin and similar penicillins containing a heteroatom in the side chain β-carbon arises from reduced interaction between the amide carbonyl and the beta-lactam group, which otherwise triggers decomposition.

**Beta-Lactamase Resistant Penicillins**

Beecham Research Laboratories introduced methicillin in 1961.5 It was the first penicillin not inactivated by penicillinases, enzymes produced by strains of staphylococci that were resistant to penicillins. These enzymes split the beta-lactam ring open, and were of several types. The Beecham team established that stability towards attack by them was achieved by placing bulky
substituents in close proximity to the labile part of the beta-lactam ring. This is described by chemists as steric hindrance. In the particular case of methicillin, ortho-disubstitution on the benzene ring gave optimal stability in the presence of beta-lactamases but antibacterial potency was reduced.

Methicillin was acid-sensitive and had to be injected. It was replaced by oxacillin in which Beecham chemists obtained steric hindrance and acid resistance by incorporating an isoxazole ring in the side chain. Four analogues were synthesised by the Beecham team, namely oxacillin, cloxacillin, dicloxacillin and flucloxacillin.

Extended Spectrum Semi-synthetic Penicillins

Unlike benzylpenicillin itself, the early semi-synthetic penicillins were not active against Gram-negative bacteria. Analysis of analogues of phenoxymethylpenicillin by Beecham chemists indicated that acid stability was enhanced by side chain substituents that attracted electrons. The most suitable substituent proved to be the amino group, the compound with this being called ‘ampicillin’. It had a wider spectrum of activity against Gram-negative bacteria than had its parent compound, benzylpenicillin, though activity against Gram-positive bacteria was somewhat reduced. These differences were due to variation in the ability of the penicillins to penetrate into bacteria. All penicillins, however, act in the same manner, namely by disrupting bacterial cell wall synthesis.

Ampicillin was an outstanding commercial success, but it had two drawbacks. The more serious was an inability to avoid destruction by bacteria capable of producing beta-lactamases, notably Staphylococcus aureus and Escherichia coli. Many microorganisms that were once sensitive to ampicillin became resistant by producing beta-lactamases. The other drawback was the polar nature of ampicillin, which reduced its ability to be absorbed from the gut. Less than half the dose was absorbed, with the result that some patients experienced diarrhoea through upset of the normal bacterial flora in the gut by unabsorbed ampicillin. This problem was largely overcome by the introduction by Beecham of the phenolic analogue amoxicillin, which had superior absorption characteristics.

A different approach to overcoming the poor absorption of ampicillin was taken by chemists at Leo Pharmaceutical Products in Denmark when they masked the polar carboxylic acid function in ampicillin to form pivampicillin. The resulting ester decomposed after absorption from the gut, liberating ampicillin. Other similar prodrugs of ampicillin were then introduced, including talampicillin and bacampicillin.
Both Pfizer and Beecham chemists reasoned that as the introduction of the basic amino group into the side chain of benzylpenicillin afforded a derivative with a wider spectrum of activity, namely ampicillin, it would be worth while to examine the effect of introducing an acidic carboxyl function. The resulting compound, carbenicillin, was obtained in 1964. It turned out to be one of the first penicillins to have high activity against *Pseudomonas aeruginosa*, a particularly troublesome pathogen that could be life-threatening in severely burned patients and in those whose immune system was compromised.

Because of the polar carboxyl group on its side chain, carbenicillin was poorly absorbed from the gut. Beecham chemists then prepared ticarcillin, which incorporated the thiophene ring previously introduced into semi-synthetic cephalosporins. Ticarcillin had strong activity against *P. aeruginosa* and so superseded carbenicillin. It is sensitive to beta-lactamases, but a combination product combining it with the beta-lactamase inhibitor clavulanic acid has been marketed.

Attempts to enhance the activity of ampicillin against Gram-negative bacteria by preparing simple N-acylated analogues resulted in a reduction in potency, though bulkier acyl groups were more promising. When the ureidopenicillin known as ‘azlocillin’ was synthesised by Bayer chemists at Elberfeld in 1971, it proved to be a broad-spectrum antibiotic. It was one of the most effective analogues of ampicillin for use against *Pseudomonas* infections, being
even more active than ticarcillin, but it and other ureidopenicillins lack resistance to beta-lactamases.\textsuperscript{12}

\[ \text{azlocillin} \quad \text{piperacillin} \]

Piperacillin is a broad-spectrum ureidopenicillin which is injected for the treatment of severe infections, especially pseudomonal septicaemia, or for peri-operative prophylaxis.\textsuperscript{13} It is synergistic with aminoglycoside antibiotics and hence is often administered concurrently with one of them.

\[ \text{6}\beta\text{-dimethylformamidopenicillanic acid} \]

During an attempted synthesis of penicillin analogues with variant ring systems, 6\beta-dimethylformamidopenicillanic acid was prepared at Leo Laboratories as a chemical intermediate. Although the synthesis of novel ring systems was unsuccessful, the novel intermediate was routinely screened. Unexpectedly, it was found to be an active antibacterial agent. A large number of analogues of it were then synthesised and tested, resulting in the introduction of mecillinam (amdinocillin).\textsuperscript{14} It was highly effective by the parenteral route against severe infections caused by Gram-negative enteric bacteria, including salmonellae, though ineffective against pseudomonal infections. The prodrug pivmecillinam was introduced at the same time as mecillinam.\textsuperscript{15} Masking of the polar carboxyl group enhanced lipophilicity, with a consequent enhancement of gut absorption. It is principally used to treat urinary tract infections.

\section*{\textbf{\beta-Lactamase Inhibitors}}

Pfizer chemists devised a method of removing the amino group from 6-aminopenicillanic acid and then used the product to form the \textbeta-lactamase inhibitor sulbactam.\textsuperscript{16} It had only weak intrinsic antibacterial activity and inhibited a narrower range of \textbeta-lactamases than did clavulanic acid.\textsuperscript{17}
Sulbactam was formulated in combination with ampicillin in both oral and parenteral products for use against organisms that had become resistant to ampicillin. These products were withdrawn from the United Kingdom market.

Tazobactam is an analogue of sulbactam that was developed by Taiho Pharmaceuticals of Tokyo. It is effective against similar β-lactamases to those inhibited by clavulanic acid, but it also inhibits those from the enterobacteriaceae. It is combined with piperacillin in a parenteral formulation that is active against organisms otherwise resistant to piperacillin.

CEPHALOSPORIN ANALOGUES

Edward Abraham and Guy Newton at Oxford were able to prepare 7-aminocephalosporanic acid from cephalosporin C in 1961, but only in low yield. By analogy with 6-aminopenicillanic acid, it was then feasible to prepare a range of novel semi-synthetic cephalosporins. This became a reality the following year when Robert Morin and his colleagues at the Lilly Research Laboratories developed a method for obtaining good yields of 7-aminocephalosporanic acid, which enabled them to synthesise cephalothin. This was the first semi-synthetic cephalosporin to be marketed. Like other so-called ‘first-generation’ cephalosporins subsequently developed, it had potent activity against Gram-positive bacteria, but was only mildly effective against Gram-negative bacteria. As cephalothin was poorly absorbed from the gut, it had to be injected. Frequent injections or continuous infusion was necessary because the acetoxy ester rapidly hydrolysed in the presence of plasma enzymes. This liberated a hydroxyl group that spontaneously reacted with the nearby carboxyl group to form an inactive lactone.

Lilly Research Laboratories next incorporated the phenylglycine side chain that Beecham Research Laboratories had previously found to confer acid stability on the beta-lactam ring of ampicillin. For cephalosporins, this also conferred some resistance to beta-lactamases as well as enhancing the activity against Gram-negative bacteria at the expense of Gram-positive activity. These changes were first seen in cephaloglycin, an orally active drug that required frequent dosing because of the presence of the metabolically labile acetoxy ester. This particular problem was overcome simply by removing the acetoxy ester, as first seen when Lilly introduced cefalexin in 1967. Although less potent than cephaloglycin, it was the first cephalosporin to be completely absorbed after oral administration. Takeda Laboratories subsequently developed cefadroxil, the phenolic analogue of cephalexin, akin to the conversion of ampicillin to amoxicillin.
At Oxford in 1961, Abraham and Newton had not only discovered that in aqueous solution the acetoxyl group of cephalosporin C could be displaced by pyridine but also that this modification altered the antibacterial spectrum. Glaxo took advantage of this to prepare cephalexin, which had a longer duration of action than cephalothin. Unfortunately, this change introduced a degree of nephrotoxicity.

Kariyone and his colleagues at the Fujisawa Pharmaceutica laboratories in Osaka subsequently found that heterocyclic thiol-containing compounds could also displace the acetoxyl group. This led to their development of cefadroxil, which incorporated a metabolically stable heterocyclic thiol that prolonged the plasma half-life and hence blood levels after parenteral administration. Despite being more potent than cephalothin when injected, cefadroxil was poorly absorbed after oral administration and had disappointing activity against Gram-negative infections.

Second-generation Cephalosporins

The ‘second-generation’ cephalosporins were characterised by a broadened spectrum of activity and a reduced tendency to be deactivated by beta-lactamases when compared with earlier cephalosporins. The methyltetrazole–thiomethyl moiety of cefadroxil proved superior to the thiadiazole–thiomethyl of cefadroxil, not only with regard to enhancement of plasma half-life but also for antibacterial potency. However, as cefadroxil lacked a phenylglycine side chain, it was inactive by mouth.
The 3’-carbamate ester in Glaxo’s cefuroxime ensured its metabolic stability, with plasma half-life being enhanced and the frequency of dosing reduced. However, of much more significance was the incorporation of an α-oximino side chain to form a syn-oxime, which made it the first cephalosporin to exhibit significantly enhanced resistance towards beta-lactamases. This rendered it particularly of value in treating infections caused by Gram-negative organisms resistant to penicillins; consequently it was widely prescribed in hospitals. The only drawback preventing its wider use was that it could not be given by mouth as it lacked a phenylglycine side chain. This was addressed when Lilly marketed cefaclor in 1979. It became one of the best selling drugs in the world until its patent expired in 1992. In response, Glaxo introduced cefuroxime axetil, an acetoxethyl ester prodrug of cefuroxime. The enhanced lipophilicity conferred by the esterification favoured absorption from the gut. The intact axetil ester then underwent enzymatic hydrolysis to liberate free cefuroxime both during passage across the intestinal wall and transport to the liver.

Third-generation Cephalosporins

The so-called ‘third-generation’ cephalosporins featured a wider spectrum of activity than earlier ones. Their importance lay in their outstanding activity against specific Gram-negative pathogens, in some cases even including Pseudomonas aeruginosa. This was partly due to their resistance to beta-lactamases as they all contained the α-oximino side chain first seen in cefuroxime. Their activity against Gram-positive bacteria, however, was inferior to that of some ‘second-generation’ drugs. Furthermore, as they all lacked a phenylglycine side chain some had to be injected. This was the case for cefotaxime, developed in Japan by Takeda, and also Glaxo’s ceftazidime. The latter was of value in treating septicaemia and also had enhanced activity against P. aeruginosa. Ceftriaxone, developed by Hoffmann–La Roche, had a longer half-life, which permitted once-daily parenteral administration for septicaemia, meningitis or pneumonia.
Researchers from Toho University School of Medicine in Tokyo and the Fujisawa Pharmaceutical Company developed cefixime, the first of the third-generation cephalosporins that could be administered by mouth.\(^\text{38}\) It had a long plasma half-life, permitting once- or twice-daily dosing. Unfortunately, there was a higher incidence of gastrointestinal disturbances than with other cephalosporins or penicillins. Cefpodoxime proxetil was developed at the Episome Institute in Gunma, Japan, and found to be well absorbed after oral administration.\(^\text{39}\)
Fourth-generation Cephalosporins

The description ‘fourth-generation’ cephalosporins has been applied to those that are resistant to destruction by beta-lactamases and have high potency against not only Gram-positive as well as Gram-negative bacteria but also \( P. \text{aeruginosa} \). The first of these was cefepime, which was developed at the Bristol–Myers Research Institute in Tokyo.\(^{40}\) Cefpirome is another, which was introduced by Hoechst.\(^{41}\) Both must be given by injection or infusion.

Cefoxitin

The premise that the lack of activity of cephemycin C against Gram-positive organisms might parallel that of the closely related cephalosporin C led Merck chemists to replace the D-aminoadipoyl side chain with others that had previously widened the spectrum of activity when incorporated into penicillins or cephalosporins.

From over three hundred \( \beta \)-methoxycephalosporins that were prepared, cefoxitin emerged with dramatically increased activity against Gram-positive organisms, while retaining activity against Gram-negative bacteria and resistance to destruction by beta-lactamases. Its activity against bacteria found in the bowel has led to its use in peritonitis.

AMINOGLYCOSIDE ANALOGUES

In marked contrast to the situation with penicillins and cephalosporins, there are very few semi-synthetic aminoglycoside antibiotics. The first one of any significance was amikacin, an
analogue of kanamycin developed by the Bristol–Banyu Research Institute in Tokyo.\textsuperscript{43} It was designed to have greater resistance to bacterial enzymes that inactivated kanamycin by phosphorylating and adenylating its hydroxyl groups or acylating its amino groups. The activity is otherwise similar to that of kanamycin, and it is prescribed in the treatment of serious infections caused by organisms resistant to gentamicin.

Clindamycin is the 7-chloro analogue of lincomycin, from which it was synthesised in 1966 by Upjohn chemists Robert Birkenmeyer and Fred Kagan.\textsuperscript{44} It is active against many Gram-positive organisms, including resistant staphylococci, as well as some Gram-negative bacteria and anaerobic organisms such as \textit{Bacteroides fragilis}. As it has good tissue penetration, clindamycin has been prescribed for the treatment of staphylococcal bone and joint infections. Since it can cause pseudomembranous colitis, its use is restricted to serious infections where the organism is known to be susceptible. Pseudomembranous colitis can be fatal, especially in elderly patients.
Netilmicin is a demethylated analogue of gentamicin C₁ that was synthesised by Schering Corporation chemists in Bloomfield, New Jersey, in 1975. It had similar activity to gentamicin, but with a significantly lower level of renal toxicity, which made it the aminoglycoside of choice in elderly patients or those with renal failure.

TETRACYCLINE ANALOGUES

Liquid formulations of the tetracyclines presented a variety of problems, particularly as they had low water solubility. Dissolving them in mildly acid solution caused epimerisation at position 4, resulting in reversal of the stereochemistry of the dimethylamin substituent, with greatly diminished activity. Acid-catalysed elimination of the tertiary hydroxyl group was also a problem. For these reasons, injections had to be used as soon as possible after reconstitution of the hydrochloride salt in water. These difficulties were overcome by reacting the carboxamido group with formaldehyde and amines to form water-soluble tetracyclines such as lymecycline. This prodrug, developed in Italy in 1959 by Carlo Erba chemists Willy Logemann and Francesco Lauria, was formulated in capsules for oral administration. It liberated tetracycline in vivo.

In 1958, chemists working for Pfizer removed the tertiary hydroxyl group from tetracyclines by hydrogenolysis over a palladium charcoal catalyst. Three years later, they introduced methacycline in which the absence of the tertiary hydroxyl group enhanced both stability and lipophilicity. This was followed by doxycycline, another Pfizer tetracycline with enhanced stability. The removal of the hydroxyl group in this case also markedly increased lipophilicity, thus enhancing both intestinal absorption and renal tubular reabsorption. This ensured that doxycycline persisted for longer in the body and permitted once-daily dosing.

The stability of these 6-deoxytetracyclines towards acid permitted the use of synthetic reagents that would have destroyed other tetracyclines. This allowed novel tetracyclines to be made, but few of them had significant clinical superiority. The exception was minocycline, which was synthesised by the Lederle Division of American Cyanamid in 1965. Its increased lipophilicity enhanced its ability to penetrate into various tissues, but also permitted its entry into the central nervous system to cause nausea, ataxia, dizziness and vertigo. On the credit side, however, minocycline penetrated into the cerebrospinal fluid for the prophylaxis of meningococcal meningitis. Furthermore, it achieved a high enough concentration in tears and saliva to eliminate the meningococcal carrier state. Other tetracyclines could not do this.
Clarithromycin, the 6-\(O\)-methyl derivative of erythromycin, is a semi-synthetic aminoglycoside antibiotic that was introduced by the Taisho Pharmaceutical Company of Japan.\(^{51}\) It was designed to have enhanced acid stability through conversion of the tertiary alcohol at the 6-position to an ether, thus preventing intramolecular reaction with the ketone at the 9-position. Clarithromycin had a similar antimicrobial spectrum to that of erythromycin, though more effective against \textit{Haemophilus influenzae}. It was better absorbed from the gut, with a lower incidence of gastrointestinal side effects. It was also more potent, with a longer half-life that allowed twice-daily dosing rather than the four times a day required with erythromycin.
Roxithromycin, an ether oxime of erythromycin, was a similar acid-stable analogue with excellent bioavailability after oral administration.\textsuperscript{52} Compared with erythromycin, it had reduced \textit{in vitro} antimicrobial activity, but this was compensated by its markedly higher plasma concentrations. Roxithromycin is used in the treatment of both soft tissue and respiratory tract infections.

Gabrijela Kobrehel and Slobodan Djokic of Pliva in Zagreb patented azithromycin, a semi-synthetic analogue of erythromycin, in 1982.\textsuperscript{53,54} It contained an expanded ring system that was acid-stable. The drug was then developed in collaboration with Pfizer. Its better absorption from the gut than erythromycin, coupled with a plasma half-life of around 70 hours, permitted once-daily dosing.

Although azithromycin was possibly inferior to erythromycin against Gram-positive bacteria, it was more effective against a number of Gram-negative organisms, including \textit{H. influenzae}. Side-effects were also less troublesome than those of erythromycin, being mainly gastrointestinal.
The semi-synthetic macrolide antibiotic ivermectin was prepared in the Merck laboratories by chemical reduction of avermectin. It was a mixture consisting mainly of the product shown here, differing only from abamectin in the absence of the double bond at the 22-position.

In 1974, the World Health Organization (WHO) launched a campaign to eliminate river blindness, a disease caused by infection with *Onchocerca volvulus*, a nematode worm carried by a black fly that bred in fast-flowing rivers. When the WHO campaign began, at least 17 million people were infected in Africa and Central America, of whom hundreds of thousands had lost their sight. By use of insecticide sprays and administration of ivermectin the disease was eradicated by the end of the century. The ivermectin was supplied free of charge to the WHO by Merck. By the mid-1990s it was being taken by 65 million people to prevent reproduction of the worm. It acted by augmenting the action of the inhibitory neurotransmitter gamma-aminobutyric acid on the reproductive tract of the female nematode.

RIFAMYCIN ANALOGUES

The elucidation of the structure of rifamycin B in 1963 opened the door to a joint programme of research between Lepetit and Ciba, in which several hundred of its derivatives were prepared. A starting point was the hypothesis that the low activity of rifamycin B could be attributed to the presence of an ionisable glycolic acid function at the 4-position, rendering it highly polar and so hindering penetration through the bacterial cell wall. It was argued that the absence of the glycolic acid side chain accounted for the enhanced potency of rifamycin SV. This hypothesis was proved to be correct when Lepetit chemists in Milan synthesised several esters, amides and hydrazides that exhibited enhanced activity.

Another key strategy adopted in the synthetic programme addressed the rapid elimination of rifamycin B through biliary excretion. This was dealt with by preparing analogues with extended side chains, several of which were well absorbed after oral administration. This led to the marketing of rifamide in some countries.
Derivatives at the 3-position of rifamycin SV were also investigated, including the 3-dimethylaminomethyl compound.58 This was superior to rifamycin SV when administered by mouth, but the plasma levels varied considerably. It was also highly susceptible to oxidation, with 3-formyl rifamycin being its principal oxidation product. When this was subjected to biological testing, high levels of antibacterial activity were recorded. A series of highly potent derivatives were then prepared from 3-formyl rifamycin, including imines, hydrazones, oximes and hydrazide-hydrazones. Of these, rifampicin (also known as ‘rifampin’) was found to be the most promising.59 It was introduced in 1966 and became established as a valuable drug in the treatment of both tuberculosis and leprosy. It is now an essential component of various combinations of drug used to treat tuberculosis or leprosy. The related rifabutin has not only proved of value in the treatment of drug-resistant tuberculosis but is also useful in the prophylaxis and treatment of non-tuberculous mycobacterial infections such as Mycobacterium avium complex (MAC) infection, a common and troublesome opportunistic infection in AIDS patients.

AZOMYCIN ANALOGUES

Following the discovery that azomycin had trichomonacidal activity, researchers at the Rhône–Poulenc laboratories in Paris synthesised a variety of nitroimidazoles. One of these exhibited strong trichomonacidal activity and had a low level of toxicity.60,61 It subsequently received the approved name of metronidazole.

After oral administration, metronidazole was capable of eliminating Trichomonas vaginalis infections carried in semen and in the urine. It was the first effective drug for the treatment of trichomonal vaginitis and remains in use for this purpose. However, its antiprotozoal spectrum was wider than first anticipated. It extends to include Entamoeba histolytica, the causative organism of tropical dysentery, as a consequence of which metronidazole has become the standard oral treatment for invasive amoebic dysentery.

Metronidazole was also serendipitously found to be an effective antigiardial agent when patients who were infected with trichomoniasis and had diarrhoea due to Giardia lamblia received treatment with it.62 Hence it became the drug of choice for the treatment of giardiasis. Serendipity again played its part when it was observed that metronidazole alleviated ulcerative gingivitis, a bacterial infection of the gums.63 This led to the realisation that metronidazole also had a wide spectrum of antibacterial activity, being especially effective against anaerobic infections. It is now widely used in the management of surgical and gynaecological sepsis. Tinidazole has similar activity to metronidazole.64
Serendipity was yet again involved when Janssen researchers were screening imidazoles for chemotherapeutic activity and found that one of them induced a profound hypnotic state in rats, whether injected or administered orally. Nearly 50 analogues were then synthesised and screened. It transpired that the best as an intravenous anaesthetic was etomidate, the ethyl ester analogue of the prototype methyl ester. Preliminary studies found it to be an extremely potent, short-acting anaesthetic and it was introduced into anaesthetic practice in 1973. The duration of action was brief because esterase enzymes in the liver were responsible for its rapid metabolism to an inactive carboxylic acid, ensuring rapid postanaesthetic recovery.

After their unexpected success with etomidate, Janssen researchers developed miconazole as a broad-spectrum antifungal that was effective against dermatophyte, yeast and mould infections. Since it was not well absorbed from the gut and was rapidly metabolised in the liver as it is a highly lipophilic molecule, miconazole was unsuitable for systemic medication other than by intravenous infusion in patients with severe infections such as aspergillosis, candidiasis or cryptococcosis. Such properties, however, were advantageous when miconazole was formulated for topical application to the mouth as a gel, swallowed as tablets in the treatment of intestinal fungal infections, or applied to the skin for ringworm infections such as athlete’s foot.

Janssen researchers found that substituted imidazoles containing a dioxolane ring had some antifungal activity in vitro. By analogy with miconazole, potency was enhanced by adding a variety of aralkyl side chains while taking into account the spatial disposition of the aryl rings. Further refinement involved altering the alkyl portion of the side chain attached to the ketal by introducing a glycidyl ether fragment in its place. From the resulting compounds, the cis isomers were found to be more active than the trans isomers. Finally, ketoconazole emerged as a potent orally active antifungal agent.

Ketoconazole was the first broad-spectrum imidazole suitable for the oral treatment of systemic mycoses. Its enhanced resistance to metabolic inactivation resulted in higher plasma levels than with earlier imidazole antifungals. Nevertheless, ketoconazole was still extensively metabolised, less than 1% of an oral dose being excreted unchanged in the urine. In addition, it was not as lipophilic as the earlier drugs, hence less of it was protein bound. The greater proportion of unbound drug compensated for any shortcomings in the absorption from the gut and metabolism. Unfortunately, when given for systemic treatment, ketoconazole has sometimes proved to be a hazardous drug. Patients have died as a result of hepatoxicity produced by it.

When Janssen researchers replaced the imidazole ring of ketoconazole with a triazole ring that was less sensitive to nucleophilic attack, they obtained compounds that were more resistant to metabolic deactivation in the liver. Terconazole was the first of these triazoles to reach the clinic but, unfortunately, it caused photosensitivity reactions in some patients. Itraconazole, an orally active analogue of terconazole, was free from this problem. As it was
highly lipophilic, itraconazole was highly tissue bound and persisted in the body to form a reservoir against infection within the skin and mucosa after dosing ceased. It is given by mouth for the treatment of candidiasis and dermatophytoses, but as there is still concern about hepatotoxicity it is not administered to patients with a history of liver disease.

Pfizer researchers prepared hundreds of analogues of ketoconazole in an attempt to increase the oral bioavailability. Replacement of the imidazole ring by the metabolically more robust triazole ring, as had been done with the Janssen analogues, provided compounds that were more resistant to metabolism. When lipophilicity was lowered by replacing the dioxolane ring with a polar hydroxyl group, drug–protein binding was reduced and so antimicrobial potency was enhanced. Eventually, fluconazole was synthesised and found to be about 100 times as potent *in vitro* as ketoconazole, with good oral bioavailability. It was also unique in possessing adequate water solubility for parenteral formulation. Due to its metabolic stability, fluconazole could be administered once daily, by mouth. It also penetrated the blood–brain barrier, making it a valuable therapeutic agent if infection had spread to the central nervous system.

**ANALOGUES OF ANTHRACYCLINE ANTIBIOTICS**

Many hundreds of analogues of doxorubicin have been prepared in the hope of finding less toxic compounds. Epirubicin, in which the 4'-hydroxyl of the sugar ring was epimerised, was found to have similar cytotoxic activity to doxorubicin, but its cardiotoxicity was reduced. It has been of value in the treatment of leukaemia, lymphomas and some solid tumours. Idarubicin is an analogue of doxorubicin in which a methoxyl group has been removed from one of the aromatic rings. It has proved to be similar to doxorubicin in the clinic.
Lederle Laboratories introduced mitoxantrone (also known as ‘mitozantrone’), a synthetic analogue of doxorubicin in which the sugar ring has been eliminated. This was prepared in light of the evidence that the toxicity of the anthracycline antibiotics was probably due to the binding of their sugar rings to heart muscle. In place of the sugar ring in mitoxantrone was a moiety with a similar physicochemical nature. Dose-related cardiotoxicity still occurs, but is reversible – unlike that of the anthracyclines. Mitoxantrone is used in the treatment of breast cancer and leukaemia.

**THIENAMYCIN ANALOGUE**

The chemical instability of thienamycin was due to the basic character of its thioaminoethyl side chain. Merck researchers examined several hundred analogues before selecting imipenem, the N-formidimidoyl derivative, for clinical application. Like thienamycin itself, it was an exceedingly potent, broad-spectrum antibiotic that was resistant to beta-lactamases.

The main problems with imipenem were that it had to be administered parenterally and it was extensively degraded in the proximal tubules of the kidneys through cleavage of the beta-lactam ring by the action of the enzyme dehydropeptidase I. This rendered it useless in the treatment of urinary tract infections. However, the discovery that various synthetic acylamino-propenoates are inhibitors of dehydropeptidase I led to the marketing of a combination of imipenem with one of these inhibitors, cilastatin.

**ANALOGUES OF MONOBACTAM ANTIBIOTICS**

Richard Sykes and his colleagues at the Squibb Institute developed a general synthesis of racemic analogues of SQ 26180 and proceeded to prepare non-methoxylated analogues as well as analogues exhibiting side chains previously found effective in the cephemycin series. A diverse range of compounds were prepared with the use of sophisticated synthetic approaches, leading to the conclusion that maximum activity was to be found in side chains featuring oxime and aminothiazole systems. This culminated in the introduction of aztreonam, which
contained a side chain seen previously in cefotaxime, a third-generation cephalosporin to which its activity can be compared. The high degree of resistance to beta-lactamase was due to the presence of a 4-methyl group adjacent to the beta-lactam function, which sterically hindered enzymatic hydrolysis.

Aztreonam proved to be effective against a wide range of Gram-negative bacteria including *Pseudomonas aeruginosa*, *Neisseria meningitidis* and *Haemophilus influenzae*, but it had poor activity against Gram-positive organisms and anaerobes.

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Antibiotic Analogues


Fungi have been the source of several drugs in addition to penicillin. During the Middle Ages, tens of thousands of people died after eating bread made from rye contaminated with a fungus known as ‘ergot’, Claviceps purpurea (Hypocreaceae).\(^1\) Outbreaks of ergotism reached epidemic proportions in the rye-bread eating areas of France and Germany, death ensuing after gangrene of the limbs had set in following prolonged vasoconstriction produced by a toxic fungal metabolite. As this manifested itself as an overall blackening of the diseased extremity, superstitious minds attributed it to charring by the Holy Fire! Such was the severity of the problem at the end of the eleventh century that a religious order was established in southern France to care for the afflicted. The scourge came to be named after the patron saint of that order as ‘St Anthony’s Fire’. Not until the seventeenth century did the cause of the affliction become generally recognised, since when isolated outbreaks have occurred right up until modern times.

Early attempts to use ergot medicinally preceded the realisation that it was the cause of so many deaths. In the second edition of his Kreuterbuch, published in Frankfurt in 1582, Adam Lonicer mentioned that midwives employed Kornzapfen to hasten labour. Kornzapfen is believed to be ergot. It has been suggested that the introduction of ergot for this purpose may have come about from midwives observing that pregnant women miscarried during outbreaks of ergotism.\(^2\) European midwives continued to administer their pulvis ad partum (birth powder), but orthodox practitioners did not seriously consider the therapeutic potential of ergot until after a letter appeared in 1808 in the Medical Repository, the first scientific journal to be published in the United States. It was written by the influential physician John Stearns of Saratoga County, New York State, who described how he had been using ergot to hasten prolonged labour for several years since being told about it by an old woman from Germany.\(^3\)

On 2 June 1813, 100 members of the Massachusetts Medical Society heard Oliver Prescott deliver his Dissertation on the Natural History and Medicinal Effects of the Secale cornutum, or Ergot (note: Secale cornutum is the Latin name for ergot of rye). This was published later that year in pamphlet form, being reprinted in London and Philadelphia and then translated into French and German.\(^4\) This finally ensured the general acceptance of the drug by the medical profession. At first, enthusiastic practitioners ignored Stearn’s warning to avoid administering ergot if prolongation of labour was due to obstruction. Many stillbirths and maternal deaths were reported.

Another use for ergot was found when the English physician Edward Woakes introduced it for treating migraine in 1868.\(^5\) It was one of many remedies for the relief of migraine proposed in the nineteenth century, but it was well received because there was a theoretical basis for its use, namely the postulated effect of its vasoconstrictive properties on swollen blood vessels in the brain.\(^6\)
Ergotamine

Ergot presented chemists with a formidable challenge. French pharmacist Charles Tanret isolated an alkaloid called ergotinine in 1875. Disappointingly, it proved to be inert. The first pure alkaloid that was active was obtained after the Sandoz Company of Basle entered the field of pharmaceutical research in 1917 by appointing the eminent Swiss chemist Arthur Stoll as its director of research. By 1920, he had isolated ergotamine. It was not until 1951 that he and Albert Hofmann were able to establish its full chemical structure. Ten years later, Hofmann achieved its total synthesis.

Ergotamine was initially marketed as a uterine stimulant, but was found to be inferior to ergot preparations. However, Sandoz pharmacologist Ernst Rothlin administered ergotamine to a patient with intractable migraine, in 1925. Encouraged by the outcome, he persuaded the eminent Zurich psychiatrist Hans Maier to evaluate its use in the treatment of migraine. This soon became the principal therapeutic application of ergotamine. However, it was a hazardous drug, with highly variable absorption after oral administration, increasing the risk of vasoconstriction and thus causing gangrene in the extremities. The maximum dose by mouth in an attack of migraine was established as 8 mg, with not more than 12 mg being taken in the course of one week. It could not be administered more than twice monthly, ruling out any possibility of giving ergotamine to prevent migraine.

Ergometrine (Ergonovine)

At University College Hospital, London, John Moir devised a sensitive method of measuring uterine contraction by inserting a small balloon in the uterus of patients undergoing routine pelvic examination one week after giving birth. The balloon was connected to a manometer that indicated the slightest pressure change. Moir used this apparatus to investigate the controversial liquid extract of ergot that many authorities had decried on the grounds that its method of preparation precluded the presence of any alkaloids known to be active. Moir unexpectedly found that the response when the extract was given by mouth was unprecedented, both in the intensity of the contractions and the rapidity of their onset. Harold Dudley, the chief chemist at the National Institute for Medical Research, then joined him to isolate the active substance in the extract. They obtained pure crystals of ergometrine in 1935. It was now clear that the traditional actions of ergot on the uterus had been due to ergometrine.
A few weeks before the publication of the British work, Morris Kharasch and his colleagues in Chicago reported their isolation of an active substance from ergot.\textsuperscript{15} This was later shown to be ergometrine, although the Chicago workers had concluded that it was not an alkaloid. Shortly after, Marvin Thompson at the University of Maryland published details of his extraction method, the patent rights to which were acquired by Eli Lilly and Company.\textsuperscript{16} The Sandoz group were also successful, with Stoll and Burckhardt publishing their results around the same time.\textsuperscript{17} Because each of these groups gave a different name to the new alkaloid, the American Medical Association felt it necessary to adopt the name `ergonovine', whereas the *British Pharmacopoeia* accepted the name `ergometrine'.

**Ergotoxine**

At the Wellcome Research Laboratory in London in 1906, George Barger and Francis Carr isolated an amorphous powder from an ergot extract.\textsuperscript{18} Henry Dale found that while it exhibited the actions of ergot, it was more toxic. This resulted in it being called `ergotoxine'. After ergotamine had been introduced into the clinic, so too was ergotoxine because of the close similarity of its actions. Despite its lower cost of production, ergotoxine fell out of popularity because of the increased risk of causing gangrene.

For over 30 years, it was believed that ergotoxine was a pure alkaloid until Albert Hofmann in the Sandoz laboratories began refining it so that he could prepare lysergic acid from it for his synthetic work (discussed in the next chapter). He began to doubt that it was a single substance and soon established that it was a mixture consisting mainly of ergocornine, together with some ergocristine and ergocryptine.\textsuperscript{19}

![Ergocornine](image1.png)

![Ergocristine](image2.png)

![Ergocryptine](image3.png)

**The Statins**

The ergot alkaloids were not the only important pharmacodynamic agents to be isolated from fungi. In 1976, two groups independently isolated mevastatin from fungi. Akira Endo and his colleagues at the Sankyo Company in Tokyo obtained it from *Penicillium citrinum* after having screened over 8000 microbial extracts for evidence of inhibition of sterol biosynthesis.\textsuperscript{20,21} Beecham Research Laboratories in England isolated it from *Penicillium brevicompactum* and named it compactin.\textsuperscript{22} Mevastatin was subsequently synthesised in the laboratory.\textsuperscript{23}
Pharmaceutical companies had begun to show interest in regulating sterol biosynthesis as a consequence of the findings from a long-term epidemiological study in which the health of the 5000 or so residents of Framingham, Massachusetts, has been closely monitored since the 1950s. The objective of this unique investigation established by the United States Public Health Service was to determine which biological and environmental factors may have been responsible for the rise in cardiovascular disease since the 1930s. One of the major outcomes of the Framingham study was the recognition of the relationship between high cholesterol values and cardiovascular disease.

Mevastatin was shown by Endo to inhibit the key enzyme that regulates hepatic synthesis of cholesterol, where at least 60% of the cholesterol in the body is biosynthesised from acetyl–coenzyme A (CoA). The enzyme is 3-hydroxy-3-methylglutaryl (HMG)–CoA reductase, which catalyses the rate-limiting conversion of 3-hydroxy-3-methylglutaryl–CoA to mevalonic acid.

The administration of mevastatin was shown to lower cholesterol levels in the liver, causing more low-density lipoprotein (LDL) cholesterol receptors to be expressed. Circulating LDL cholesterol levels then fell due to the increased uptake of LDL cholesterol by the greater number of hepatic LDL cholesterol receptors.24

Endo also isolated lovastatin (which was originally called ‘monacolin K’) from the filamentous fungus Monascus ruber.25 It was also obtained from the fungus Aspergillus terreus by researchers at Merck and Company (and named by them as ‘mevinolin’), who confirmed that it was a potent inhibitor of HMG–CoA reductase.26 It was more potent than mevastatin as an inhibitor of sterol biosynthesis and has become one of the most widely prescribed drugs in the world, as have other ‘statins’. The natural and semi-synthetic statins now on the market have had a worldwide impact on heart disease, with the full implications of their use in people with elevated cholesterol levels only now becoming fully appreciated.

**DRUGS FROM ACTINOMYCETES**

During the 1950s, Ciba researchers led by Hans Bickel isolated from Streptomyces pilosus several iron-containing antibiotics known as ‘ferrimycins’. Their antibiotic activity was
disappointing as bacterial resistance quickly developed, but in addition the activity was inhibited by contaminants that contained iron. The major contaminant was sent to Victor Prelog at the Swiss Federal Institute of Technology in Zurich, where it was identified and named ‘ferrioxamine B’.27

After animal tests had confirmed its safety, ferrioxamine B was supplied to Woehler at the University Hospital in Freiburg for evaluation in patients with iron deficiencies.28 The first patient received an intravenous injection and was somewhat distressed when he shortly after passed urine coloured deep reddish brown. This was due to rapid and total elimination of unmetabolised ferrioxamine B by the kidneys. Woehler realised that was unique, all other known iron compounds breaking down and releasing their iron in the body. He then postulated that if iron could be removed from the ferrioxamine B, the residue might be a strong iron acceptor that was capable of removing excessive iron from the body in diseases involving iron overload.

Bickel was able to prepare the compound Woehler wanted, namely desferrioxamine (also known as ‘deferoxamine’).29 It was injected into animals intravenously and found to possess a very high affinity for ferric iron, as evidenced by the presence of iron in the urine. Further investigation revealed that iron in haemoglobin and in enzymes was not affected by the drug. This permitted its administration to a patient with severe haemochromatosis, a condition in which excessive amounts of iron are absorbed from dietary sources. Repeated injections were required, but that patient and several others were successfully treated during the next few months.28 So, too, were two patients with thalassaemia, a genetic disorder of haemoglobin synthesis. Young patients given repeated blood transfusions frequently suffered life-threatening cardiac and renal complications from the consequent iron overload. Removal of excess iron by desferrioxamine radically transformed their lives. A subsequent clinical trial at the Hospital for Sick Children in London resulted in desferrioxamine being accepted as the standard chelating agent for treatment of iron overload in thalassaemia.30

**Acarbose**

Acarbose was isolated in 1975 from strains of *Actinoplanes* spp., actinomycetes found in cultures containing glucose and maltose. It was synthesised in the laboratory in 1988.32

Acarbose was found to be a reversible inhibitor of alpha-glucosidase, an enzyme that breaks down disaccharides, trisaccharides and oligosaccharides in the gut into glucose.33 It was realised that such an alpha-glucosidase inhibitor might interfere with the digestion of dietary starch and sugar. A clinical trial in non-insulin-dependent diabetic patients found that it slowed the digestion of polysaccharides and sucrose and thereby reduced the rise in plasma
glucose levels after meals. Acarbose is currently used for this purpose, either on its own or with oral antidiabetic drugs.

**DRUGS FROM BACTERIA**

Streptokinase was the first fibrinolytic agent to be employed in the clinic. It was isolated from cultures of β-haemolytic streptococci in 1945 by researchers working under the guidance of William Tillett at New York University Medical School and later purified in Lederle’s laboratories. Its amino acid sequence was established in 1982.

Fibrinolytic drugs act by increasing the amount of plasmin in the blood. Plasmin is present in blood to prevent unwanted clotting by catalysing the breakdown of the fibrin polymer that constitutes the framework of a blood clot. The plasmin is formed from plasminogen, a process that occurs once plasminogen has been activated by complexing with fibrin. The entire process is carefully balanced, but fibrinolytic drugs can tilt the balance to increase plasmin formation.

Streptokinase administered intravenously reacts with uncomplexed plasminogen in the blood. The resulting streptokinase–plasminogen complex behaves in much the same way as the natural fibrin–plasminogen complex and converts uncomplexed plasminogen to plasmin. Thus the circulating levels of plasmin are increased and fibrinolysis occurs, dissolving intravascular blood clots.

The first major clinical application of streptokinase was in the dissolution of pulmonary embolisms. As more experience was acquired, 80–90% success rates in dissolving these clots were achieved. Streptokinase was next used to dissolve the occlusive clots in the coronary artery that are the cause of acute myocardial infarction, the commonest cause of death in industrialised countries. Large-scale clinical trials confirmed that streptokinase or other fibrinolytic drugs can reduce mortality by about one-quarter, thereby ensuring that this has become the initial means of treating such patients. Combination treatment with a fibrinolytic drug and low-dose aspirin doubled survival rates.

Beecham laboratories prepared their semi-synthetic fibrinolytic agent anistreplase from human plasminogen and streptokinase. The site responsible for catalysing plasmin formation from free plasminogen was blocked with a 4-methoxybenzoyl group (anisoyl group), effectively converting the complex into a prodrug that slowly deanisoylated in the blood to release the active plasminogen–streptokinase complex. The slow decomposition to the active complex meant that it did not need to be administered by continuous infusion for one to three days, as had been the case with streptokinase. Instead, it was intravenously injected over a period of five minutes.

**Botulinum Toxin A**

The toxin produced by *Clostridium botulinum*, an organism that causes fatal food poisoning, paralyses muscle by preventing the release of acetylcholine from presynaptic nerve terminals. This relaxes muscles, including those in spasm.

Following a decade of clinical investigations, the US Food and Drug Administration in 1989 licensed a haemagglutinin complex of botulinum toxin A for treatment of certain spasmodic disorders such as hemi-facial muscle spasm or blepharospasm, a condition in which muscular spasm causes uncontrollable blinking and even total closure of the eyelids. Local injections of nanogram quantities of the toxin are administered.

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Following his discovery that ergotoxine was a mixture consisting mainly of ergocornine, ergocristine and ergocryptine, Sandoz chemist Albert Hofmann prepared hydrogenated derivatives of each of these alkaloids, and also of ergotamine. Dihydroergotamine had a similar action to ergotamine and was introduced for the treatment of migraine as it had reduced peripheral vasoconstrictor activity, which arguably rendered it less likely to cause side effects.\textsuperscript{1} After their pharmacological evaluation of dihydroergocornine, dihydroergocristine and dihydroergocriptine, Sandoz marketed Hydergine\textsuperscript{1} in 1949. It was prepared by hydrogenating ergotoxine and now has the approved name of codergocrine. It was recommended as a coronary vasodilator to increase blood flow in senile patients with cerebral insufficiency and for many years was the most popular product Sandoz sold in continental Europe. It had fallen out of popularity by the 1980s, but recently it has been recognised as having clinical value and there is a need for further controlled trials to assess its proper role.\textsuperscript{2}

Hofmann had chosen to work with ergot alkaloids even though Stoll abandoned this area once ergotamine had been introduced into the clinic. The introduction of ergometrine was one of two developments that convinced Hofmann that the moment was opportune. The other was the isolation of lysergic acid from an alkali digest of ergotinine by Walter Jacobs and Lyman Craig of the Rockefeller Institute in New York in 1934, and their subsequent recognition of it as the common component of all ergot alkaloids.\textsuperscript{3} Dihydrolysergic acid was synthesised in 1945 by Frederick Uhle and Jacobs,\textsuperscript{4} but it was not till 1954 that Robert Woodward and his colleagues synthesised lysergic acid.\textsuperscript{5}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{lysergic_acid.png}
\caption{Lysergic Acid Derivatives}
\end{figure}

Lysergic acid had no pharmacological activity, but Hofmann successfully exploited it for the preparation of several clinically important semi-synthetic analogues. He began by making ergometrine from it in 1937, thereby opening the way to an economic route to it via lysergic acid from ergotoxine. He next prepared a series of ergometrine analogues.\textsuperscript{6} Among these were methylergometrine, which was at first thought to be superior to ergometrine for obstetric use, but is no longer available in the United Kingdom.
Another of the analogues prepared by Hofmann was one that was designed to enhance the activity of dihydroergotamine as a cerebral dilator for the elderly. In it, Hofmann incorporated molecular features found in nikethamide, a respiratory stimulant. The resulting molecule was code-named LSD-25 as it was the twenty-fifth lysergic acid derivative prepared by Hofmann. It appeared unsuitable for clinical use as it caused excitement in some animals and a cataleptic condition in others, but Hofmann re-examined it in the spring of 1943 because it was a powerful uterine stimulant. He was forced to stop work one afternoon because of dizziness and a peculiar restlessness. This was followed by extraordinary hallucinations for almost two hours. After recovering, Hofmann realised that the LSD-25 might somehow be responsible for his experience, even though he could only have ingested minute traces of it by accident. Subsequent self-experimentation confirmed that LDS-25 was an exceedingly potent psychotomimetic agent. A detailed study was then carried out at the psychiatric clinic of Zurich University, demonstrating that a few micrograms of LSD-25 taken by mouth could cause profound alterations in human perception.

The subsequent discovery by John Gaddum at Oxford that LSD-25 was a potent, selective 5-HT antagonist stimulated worldwide research into the role of neuroamines in the brain. Despite extensive investigations, however, no accepted role for LSD-25 in medical or psychiatric practice has yet been found, although it has been employed by military agencies for brain washing. Its illicit use since the 1960s as a recreational agent is well known. In medical circles it is now referred to by its approved name of ‘lysergide’.

In 1957, Hofmann initiated further studies on psychotomimetic compounds related to lysergide after he received samples of mushrooms held sacred by Mexican Indians, who called them teonanácatl (flesh of God). These had been collected by Gordon Wasson, a retired financial journalist from New York. Together with his wife Valentina, he had made a life-long study of the role of mushrooms in human society. He had uncovered the story of the cult centred around ‘magic mushrooms’, which were categorised by the botanist Roger Heim as Psilocybe mexicana. Hofmann isolated crystals of psilocybin from these mushrooms, which was an indole alkaloid – as was lysergide. He swallowed it himself and found that it produced hallucinations.
Hofmann next investigated *ololiuqui*, an Aztec magical plant still used by Mexican Indians in religious rituals. Wasson had also brought it to his attention. Hofmann identified it as the seeds of two types of morning glory, viz. *Rivea corymbosa* (L.) Hall and *Ipomoea violacea* L. On analysis in 1960, these were unexpectedly found to contain lysergide and alkaloids closely related to it, including ergometrine. In 1976, Wasson speculated that ergot might have been the hallucinogen that the Ancient Greeks employed in their celebration of the Mysteries at Eleusis. Hofmann knew that Ernst Chain and his colleagues in Rome had demonstrated that hallucinogenic lysergic acid derivatives were present in *Claviceps paspali*, a variety of ergot that often infected a wild grass growing around the Mediterranean basin. Hofmann confirmed that the same alkaloids were present in ergot grown on wheat or barley as were to be found in that grown on rye – an important point so far as Wasson’s speculation about the Eleusian Mysteries was concerned, for the Ancient Greeks did not cultivate rye. Hofmann realised that the only alkaloid readily extracted from ergot by water, as must have been the case in the Mysteries, was ergometrine. Once again, his self-experimentation revealed that it was hallucinogenic, but this raised the question of why this had not been previously discovered. His explanation for this was that its effects on the uterus were exerted by an oral dose of less than one quarter of a milligram, whereas the hallucinogenic dose was in the order of 1–2 mg. This lent support to Wasson’s contention that ergot was used in the celebration of the Eleusian Mysteries, but it also explained the occurrence of madness in many victims of ergotism in the Middle Ages. It is difficult to pass judgement on Wasson’s assertions, although they have certainly been accepted in some quarters. The same is true of the claim that the bizarre events surrounding the Salem witchcraft trial in New England may have been associated with the consumption of contaminated rye.

**Methysergide**

Apart from Hofmann’s studies on hallucinogenic alkaloids, Sandoz investigators continued to exploit the medicinal potential of lysergic acid derivatives. They established that substitution on the 1- or 2-position of the indole ring enhanced potency as a 5-HT antagonist on the isolated rat uterus. The placing of a methyl group on the ring nitrogen of ergometrine, for example, increased antagonist activity by a factor of 24. Variation in the amide substituent led to the synthesis of methysergide, which was much more potent than lysergide as a 5-HT antagonist and hence was tested for its ability to prevent migraine.

![Methysergide](image)

Although an effective migraine remedy, methysergide was subsequently found to have dangerous side effects, including fibrosis of the heart valves and retroperitoneal fibrosis. Consequently, it has been reserved for the prophylaxis of migraine in patients who are severely incapacitated by migraine attacks.

**Bromocriptine**

At the Weizmann Institute of Science in Israel, Moses Shelesnyak began a search for a drug that could interfere with the uterine deciduoma reaction that was associated with ovum
implantation in rats. In 1954, he reported that ergotoxine was active and attributed this to its inhibition of prolactin secretion. This effect could be reversed by injections of either progesterone or prolactin, indicating that ergotoxine acted via the hypothalamus and pituitary to inhibit prolactin secretion. This work by Shelesnyak opened up a new area of research in the field of the ergot alkaloids. The immediate outcome was that Sandoz researchers initiated a search for an ergot analogue that selectively inhibited prolactin secretion. This led to their development of the 2-bromo derivative of ergocriptine, namely bromocriptine.

While using bromocriptine to inhibit prolactin secretion during an investigation into the role of dopamine-secreting neurones in the hypothalamic–pituitary axis, Swedish investigators discovered that it had a dopamine-like action. As deficiency of dopamine was associated with the occurrence of Parkinson’s disease, it was examined as a supplement to levodopa treatment. Clinical trials confirmed its value in severe cases, but as a major side effect was involuntary movement, bromocriptine is reserved for patients who have ceased to respond to levodopa.

**Pergolide**

In 1949, Hofmann reduced the methyl ester of lysergic acid with lithium aluminium hydride and obtained lysergol. This compound was later isolated from *ololiuqui* and found to be one of its less psychoactive constituents insofar as oral doses of 8 mg merely induced slight sedation in volunteers.

Lysergol has been a valuable intermediate in the preparation of semi-synthetic analogues since its tosylate or mesylate heated with a thiol compound in a polar solvent readily underwent nucleophilic displacement, thereby introducing a sulfur-containing side chain. Pergolide was prepared in this manner by Lilly researchers and shown to be a potent dopamine receptor agonist. It was then introduced as an adjunct to levodopa therapy in a similar manner to that of bromocriptine.

**Quinagolide**

Sandoz chemists Rene Nordmann and Trevor Petcher realised that, as both ergot alkaloids and apomorphine were dopamine agonists, it might be possible to postulate a common molecular moiety and base a simplified ergot alkaloid structure on this to obtain a novel
dopamine agonist with high specificity of action. They then synthesised a compound that retained key structural features of apomorphine and the dihydrogenated ergot alkaloid pergolide and also an experimental drug known as CQ 32-084.

In order to avoid rapid metabolic deactivation of a catechol system, only a single phenolic group was included in the planned compound. This led to the development of racemic quinagolide in 1984. It combined the oral activity, high potency and long duration of action of the ergot alkaloids, with the higher specificity of action of apomorphine as a dopamine agonist. Further investigation revealed that the dopaminomimetic activity resided solely in the (−)-enantiomer, which proved to be a highly selective dopamine D2 receptor agonist. In rats, quinagolide inhibited prolactin secretion without affecting levels of other pituitary hormones and it inhibited the growth of pituitary tumours. In the clinic, it was effective in reducing prolactinaemia in patients with prolactin-producing pituitary tumours.

**SEMI-SYNTHETIC STATINS**

Pravastatin is an active metabolite of mevastatin. It was much less lipophilic than mevastatin and hence was less likely to penetrate extra-hepatic cells by passive diffusion. This meant that pravastatin was free of some of the side effects that arose with mevastatin. It could be given once daily by mouth in the management of hyperlipidaemia.

When Merck researchers introduced an additional methyl group adjacent to the ester function in the side chain of lovastatin to form simvastatin, they found that this more than doubled the potency as an inhibitor of HMG–CoA reductase. Simvastatin was the first HMG–CoA reductase inhibitor for the treatment of severe hyperlipidaemia to be introduced into the clinic in the United Kingdom, shortly before pravastatin was marketed. There was little to choose between them. Several other statins have been introduced since then.

**REFERENCES**

August Wilhelm von Hofmann distilled aniline from coal tar in 1843 while working in Giessen as a research student with Justus Liebig. Two years later, he moved to the Royal College of Chemistry in London, where he demonstrated that benzene was present in coal tar. One of his students, Charles Mansfield, subsequently isolated it by fractional distillation of the tar. Nitration of benzene with nitric acid then provided the basis of a route to the industrial manufacture of aniline dyes and other important organic chemicals.

In 1856, another of Hofmann’s students, William Perkin, oxidised the aniline derivative allytoluidine in an overly ambitious attempt to synthesise quinine. Instead he obtained a dark substance that turned fabrics purple. This was the first synthetic dyestuff, which Perkin initially called aniline purple but later changed to mauveine. Realising the commercial value of the dye, Perkin established his own factory the following year. This marked the start of the synthetic dyestuffs industry that was to fuel a demand for organic chemists who could discover new products through the application of research. The high price of natural dyes was a matter of concern for the rapidly expanding textile industry, which was trying to match the demand from a growing population for cheap clothing. Perkin’s mauvine remained expensive to produce, but within a decade several manufacturers were developing a range of affordable new dyes from aniline, toluidine and quinoline. Although the industry began in England, it was in Germany that it thrived. Two factors largely accounted for this. Once the German economy had recovered from the collapse of its stock market in 1873, industrialisation entered its second phase in which the chemical and electrical industries rapidly expanded to compete in importance with the existing coal, iron and steel industries. The heavy investment in the manufacture of synthetic dyes soon put Germany well ahead of all its competitors in this field. The second factor that made this possible was the willingness of German universities in the 1870s and onwards to meet the need of the moment. German chemists rapidly became the leaders in the emerging field of organic chemistry and remained so until the outbreak of the Second World War. They wrestled with the nature of the structures of the novel molecules they had synthesised, skilfully breaking them apart to identify known fragments, and then deducing how the atoms were assembled in the intact molecules. New synthetic reactions were also introduced, providing routes to a vast range of novel dyes and other commercially important organic compounds, including synthetic drugs. Success bred success. In marked contrast to the situation in Germany, the failure of the United Kingdom to maintain the lead that Perkin had given it with mauvine was in no small measure due to the disdain with which its universities at the time viewed industrial contacts.

The German pharmaceutical industry developed directly out of the dyestuffs industry when leading manufacturers like F. Bayer & Company and Farbenfabriken Hoechst realised that their chemists could produce medicines as well as dyes. Initially, a few dyes served as drug prototypes, but during the twentieth century the industry became completely independent of its origins and instead concentrated on chemically modifying the structures of natural
products from plant or biochemical sources. In Britain, France, the United States, Canada and to a much lesser extent Switzerland, the industry continued to focus on the extraction of alkaloids and glycosides from plants, with only a minimal effort being expended on the development of synthetic drugs. Such an approach was still capable of bringing immense benefits to the sick, as illustrated by the isolation of insulin in Canada and penicillin in the United Kingdom. However, a gradual change of direction in favour of synthetic drugs came about because of shortages during the two World Wars of essential medicines normally supplied by Germany.

The majority of natural products, be they from the plant or animal kingdoms, have been isolated in academic laboratories. However, the opposite is true of synthetic drugs. With the exception of a handful of hypnotics, the first synthetic drugs were all developed in industrial laboratories or research institutes where the raison d’être was the development of new medicines, such as the Institute for Experimental Therapy established by Paul Ehrlich in Frankfurt.

PHENOL

When the inventor William Murdock first used coal gas in 1794 to illuminate his home in Redruth, Cornwall, he could not have envisaged the full consequences of his actions. Within seven years, buildings in Birmingham were being lit by gas and before long the streets of other major British cities were no longer dark at night thanks to locally produced gas. In the United States, gas lighting had been installed in Baltimore by 1817. There was, however, one unwelcome by-product that arose from this exciting development, namely vast amounts of apparently worthless coal tar. One of the first to examine it was Friedlieb Runge, the chief chemist of a gas works at Oranienburg near Berlin, who steam-distilled a light oil from it. A portion of this oil was acidic and so dissolved in milk of lime. Runge gave the name carbolic acid to the material he then recovered by acidifying the lime solution. Charles Gerhardt named it phenol in 1842.

Friedlieb Runge had been impressed by the ability of carbolic acid to prevent the decay of animal tissue and wood, but felt that it would be too expensive to market it as a preservative. In 1844, however, the French physician Henri-Louis Bayard incorporated coal tar in a clay-based powder for disinfecting manure to be used as a fertiliser. This won him a prize from the Société d’Encouragement for its contribution to hygiene.

The first person to exploit the disinfectant properties of phenol was the industrial chemist Frederick Calvert, who had studied and worked in France from 1835 to 1846. Returning to Manchester, he became a consultant chemist and introduced phenol for embalming. He became closely involved with the inventors of McDougall’s Powder, a crude mixture of calcium salts and phenol patented in 1854 for purifying water and deodorising sewage. Calvert manufactured the powder and saw it become very popular as a disinfectant for stables, farmyards and any place where putrefying material was to be found. It was also applied to sores.

Calvert became convinced that the disinfectant in coal tar was phenol and accordingly informed the Académie des Sciences in 1859. This encouraged the Dresden physician Friedrich Küchenmeister to employ pure phenol as a wound dressing. Meantime, a pharmacist from Bayonne, LeBeuf, asked Jules Lemaire to evaluate his emulsified coal tar. It proved very successful in treating septic wounds and in April 1862 was authorised for wound disinfection in the civil hospitals of Paris. The following year, Lemaire’s book entitled De l’Acid Phénique was published, followed by an enlarged 2nd edition in 1865. This established Lemaire as the leading advocate of the use of phenol in surgery at that time, although his work aroused little enthusiasm in Britain.
Gilbert Declat’s lengthy volume entitled *Nouvelles Applications de l’Acide Phénique en Médecin et en Chirurgie* was also published in 1865. Declat referred to phenol as a ‘parasiticide’. In contrast to Lemaire, he was fully cognisant with Pasteur’s ideas. He expressed the hope that phenol would be used to prevent infection and even recommended washing of the walls and surroundings of the sick room with it.

British surgeons continued to ignore the developments in France. Fortunately, Calvert had convinced public authorities in Britain of the benefits of phenol for treating sewage and a newspaper report about its use in Carlisle was read by Joseph Lister, the Professor of Surgery at the University of Glasgow. Though deeply concerned about the high incidence of lethal infections following surgery, reaching 40% after amputations, he had been unaware of the studies being carried out in France with phenol. After reading the newspaper article, he applied German creosote when operating on a patient with a compound fracture of the leg. The prognosis was poor since puncturing of the skin by the broken bone had resulted in infection and Lister was unable to save the patient. He went on to modify his technique by covering wounds with dressings soaked in solutions of pure phenol obtained from Calvert. On 12 August 1865, a boy run over by a cart was admitted to the Royal Infirmary with a compound fracture of the leg. This time, the dressing successfully prevented infection. Eleven more patients were treated, with only one death. The first of several papers by Lister on antiseptic surgery then appeared in the *Lancet* in 1867. By basing his use of phenol on a clear understanding of Pasteur’s researches, Lister was highly successful in preventing wound sepsis and he transformed surgical practice and rendered it safe. Antiseptic surgery was soon replaced by aseptic surgery, itself a logical development of Lister’s approach. With the demise of antiseptic surgery, phenol became much less important. The main objection to its use was its corrosive nature, which permitted only low concentrations to be applied to the skin. Today phenol is found only in antiseptic creams and liquids such as mouth washes.

**ANALOGUES OF PHENOL**

Alternatives to phenol were sought as early as 1867 when Arthur Sansom at London’s Royal Hospital for Diseases of the Chest administered sulfocarbolate of potash by mouth in the mistaken belief that it would slowly decompose in the body to release small amounts of phenol and thereby act as an internal antiseptic. The product he used was a mixture of the salts of ortho- and para-phenolsulfonic acids, apparently consisting largely of the former. This was subsequently named solozic acid, and became available commercially as a one-in-three solution in water. It was widely used for treatment of diphtheria, scarlet fever and puerperal fever until Heinrich Bechhold and Paul Ehrlich revealed its inferiority to other phenolic compounds.

![solozic acid](image)

**Salicylic Acid**

Carl Thiersch, the Professor of Surgery at Lepizig, adopted a similar approach to Sansom in seeking a compound with less deleterious effects than phenol on tissues. As the first German surgeon to adopt Lister’s methods, he had become well aware of its damaging effects. When he discussed the matter with Hermann Kolbe, the Professor of Chemistry and by now the leading
chemist in Germany, the latter recalled how in 1860 he and Lautemann had treated phenol with carbon dioxide in the presence of sodium under pressure to form salicylic acid. Kolbe knew that, on heating salicylic acid, carbon dioxide was liberated and the acid decomposed into phenol, so he now carried out some simple tests on salicylic acid and confirmed that it had antiseptic properties. His idea that it might release phenol was never realised, but salicylic acid did find a role as an antiseptic that was somewhat less corrosive than phenol. That it was still damaging to tissues is evident from its continued use to burn out warts. Convinced of the value of salicylic acid as a substitute for phenol, Kolbe modified his original synthesis so that the acid could be produced on an industrial scale. One of his former students then opened the Salicylsa¨urefabrik Dr F. von Heyden in Dresden, as a consequence of which salicylic acid became cheaply available in 1874.

Although it never replaced phenol in surgical practice, salicylic acid became popular as an internal antiseptic at a time when it was widely believed that many diseases arose from the presence of pathogenic bacteria in the gut. This led Carl Buss at St Gallen in Switzerland to administer salicylic acid by mouth to typhoid patients. When the course of their disease was routinely checked by thermometry, it became obvious that salicylic acid was an effective antipyretic. However, it did not lower body temperature by curing the typhoid infection. Widespread interest was aroused in 1875 when Buss published his observation that repeated doses of salicylic acid could control fevers without causing the side effects of quinine, at that time the standard antipyretic. Salomon Stricker at the University of Vienna Medical School then tested salicylic acid for its ability to reduce the temperature of patients with rheumatic fever. To his surprise, it also proved to be of definite value as an antirheumatic drug. A similar observation was made by the Scottish physician Thomas MacLagan, while the French physician Germain Sée confirmed the specific value of salicylic acid in rheumatoid arthritis and gout. Surprisingly, many physicians were unaware of these reports until the 1950s when, at last, there was universal recognition of the importance of salicylate therapy in rheumatoid arthritis.

Phenyl Salicylate

Salicylic acid was normally prescribed as its sodium salt. Many patients complained about its unpalatability and irritating effects on the stomach. An attempt to improve upon both it and phenol as internal antiseptics was made by the Polish chemist and physician Marceli Nencki in 1883, when he reacted the two drugs together to form phenyl salicylate. After being swallowed, this passed unchanged through the stomach because it was highly insoluble. It was more soluble in the small intestine, where the portion that dissolved then decomposed to liberate small amounts of the parent drugs. After Hermann Sahli had tested phenyl salicylate in Berne, it was generally believed that some benefit was to be derived from these small amounts.
Phenyl salicylate was marketed under the name ‘Salol’ and it was many years before there was general recognition that any advantage it had over salicylic acid was offset by the prolonged onset of activity and variability of therapeutic response. Until then, it was a popular substitute for salicylic acid as an antipyretic and antirheumatic. It was an early example of the gullibility of many when presented with a drug exhibiting chemical novelty unsupported by reliable clinical proof of efficacy.

Aspirin

After being appointed in 1896 by F. Bayer & Company of Elberfeld to stimulate research so as to free the company from its dependence upon universities for the supply of new compounds, Arthur Eichengrün began to prepare esters of phenolic compounds that irritated the stomach. He expected that the masking of the phenol would protect the stomach, while the esters would decompose once they reached the more alkaline conditions of the gut and release the active drug for it to be absorbed into the circulation. Felix Hoffmann was given the task of preparing acetylsalicylic acid, a crude version of which may have been synthesised in 1853 by Charles von Gerhardt. After acetylsalicylic acid had been prepared, it was tested in the spring of 1897 by the company pharmacologist Heinrich Dreser. He rejected it – despite the tests appearing to Eichengrün to show that it was superior to any other salicylate. Acting on his own initiative, Eichengrün tested the compound on himself, then arranged for it to be clandestinely evaluated by physicians in Berlin. The outcome of this was not only to confirm that acetylsalicylic acid was an effective substitute for salicylic acid, but also that it had unexpectedly relieved pain when a patient with toothache happened to be given a sample to consume. Once the analgesic properties had been confirmed in other patients, the colleague who had conducted the secret trials brought this to the attention of Bayer management. They responded by arranging for Kurt Witthauer of the Deaconess Hospital in Halle and Julius Wohlgemuth in Berlin to conduct independent clinical trials of the drug. The outcome persuaded Bayer management to market acetylsalicylic acid under the proprietary name Aspirin, which was coined by Eichengrün from ‘a’ for acetyl and ‘spirin’ from *Spirea ulmaria*, the now obsolete name of the plant from which salicin was obtained. To ensure the success of the new drug, F. Bayer & Company circularised more than 30,000 doctors in what was probably the first mass mailing of product information. Eichengrün was rewarded for his efforts by being promoted to Director of Pharmaceutical and Photographic Research, while Hoffmann became Director of Pharmaceutical Sales.

Dreser was asked by the Bayer management to publish the results of his further examination of aspirin after its testing in Berlin, in order to lend scientific credibility to the new product. His paper omitted any reference to either Eichengrün or Hoffmann and gave no indication of how aspirin came to be developed. The first account of this did not appear until a year after the Nazi party came to power in Germany in 1933. It was published in a history of chemical engineering as a short footnote that claimed to be based on a communication from Felix Hoffmann to the author. This alleged that when Hoffmann had been asked by his rheumatic father to find an alternative to the foul-tasting sodium salicylate, he searched the literature and came across acetylsalicylic acid, then preparing it in pure form. On the fiftieth anniversary of
the introduction of aspirin, Arthur Eichengrün published the only detailed account ever written by any of those directly involved in the development of aspirin. In this, he implied that history had been rewritten by the Nazis to hide the fact that it was a Jew who was primarily responsible for the development of the most famous drug in history. This appears to have been as unpalatable to some as sodium salicylate was supposed to have been for Hoffmann’s father, leaving the present writer to attempt to set the record straight on the centenary of the introduction of aspirin.

How aspirin worked remained a mystery until 1971 when John Vane at the Institute of Basic Medical Sciences of the University of London and the Royal College of Surgeons of England demonstrated that it blocked prostaglandin synthesis. The precise manner in which this occurred was subsequently shown to be through the permanent transfer of the acetyl group from aspirin on to the hydroxyl group of a serine residue located 70 amino acids from the C-terminal end of the cyclooxygenase (COX) enzyme that promotes the formation of prostaglandins.

Another effect of aspirin that has been successfully exploited is its antiplatelet activity, which also arises from blocking of prostaglandin synthesis. In 1949, Gibson described his successful use of aspirin in a small group of patients with vascular problems. Around the same time, Lawrence Craven in California realised that his tonsillectomy patients who had taken a chewable aspirin preparation for pain relief were more likely than others to bleed. Craven went on to conduct an uncontrolled investigation on 8000 patients who regularly consumed aspirin and claimed that none suffered heart attacks. None of his publications appeared in prominent journals and, when he died of a heart attack despite taking aspirin, any credibility his work might have carried was undermined. Fortunately, in New York in 1967, Harvey Weiss established that the prolongation of bleeding time caused by aspirin was due to an impairment of platelet aggregation. He suggested that aspirin might be an antithrombotic drug and in 1971 was able to provide experimental evidence that this was the case. He urged that clinical trials be carried out. Three years later physicians in Wales published the results of the first randomised, controlled clinical trial of aspirin in patients who had experienced a previous heart attack. Since then, it has taken many years for it to be generally accepted that low doses of aspirin reduce the risk of myocardial infarction in patients with cardiovascular disease.

Aspirin Analogues

Anthranilic acid (o-aminobenzoic acid), an analogue of salicylic acid in which the phenolic hydroxyl is replaced by an amino group, is inactive. Parke, Davis and Company developed a non-steroidal anti-inflammatory agent called mefenamic acid, by adding a second benzene ring that drastically reduced the basicity of the aromatic amino group in order to prevent zwitter ion formation. It was patented in 1961. The researchers also confirmed that flufenamic acid, which had originally been synthesised in 1948, was a useful anti-inflammatory drug. Geigy researchers subsequently developed diclofenac by taking into account the structural physicochemical characteristics of existing anti-inflammatory agents.
Diflunisal was introduced by Merck Sharp and Dohme after more than 500 compounds had been synthesised and evaluated in a 15 year long search for a longer-acting, safer analogue of aspirin.\(^3\) It is similar in its therapeutic profile to arylpropionic acid-derived non-steroidal anti-inflammatory drugs.

\[ \text{p-Aminosalicylic Acid} \]

While investigating the nutritional requirements of the causative organism of tuberculosis, \textit{Mycobacterium tuberculosis}, in 1940 Frederick Bernheim, a biochemist at Duke University Medical School in North Carolina, discovered that benzoic and salicylic acids increased oxygen utilisation.\(^4\) This indicated that these acids were serving as nutrients for the bacteria. Taking into consideration the recently announced antimetabolite theory, he went on to antagonise the effect of these acids with 2,3,5-triiodobenzoic acid.\(^4\) In conjunction with Alfred Burger and others, Bernheim then examined a diverse range of halogenated aromatic acids and phenolic ethers as potential antimetabolites. Some of the latter were active, but unsuitable for clinical application because of side effects on the central nervous system.

Bernheim had communicated his findings to his friend Jorgen Lehmann at the Sahlgren’s Hospital in Gothenburg. Reflecting on the past at the age of 83, Lehmann has written that in 1943 he was convinced that the positioning of the amino group in the sulfanilamide antagonist \(p\)-aminobenzoic acid was critical; hence he felt that a \(p\)-amino group should be introduced into salicylic acid to provide a tuberculostatic drug.\(^4\)

Lehmann asked the Ferrosan Company of Malmo (now incorporated into Kabi Pharmacia) to supply him with \(p\)-aminobenzoic acid as it had previously been prepared only in quantities insufficient for biological evaluation. As a result, he was able to test it in January 1944 and found it to be tuberculostatic in animals, with a wide margin of safety. In March of that year, a child with a severely infected wound was successfully treated by local application of the drug. By the end of the year, 20 patients had received the drug by mouth and results were most promising. Lehmann published the result of two years of clinical trials, confirming that \(p\)-aminosalicylic acid (PAS) could cure tuberculosis.\(^4\)

It was later established that \(p\)-aminosalicylic acid was best used in combination with the much more potent drugs streptomycin and isoniazid. The combination of these drugs proved to be a major step in overcoming the problem of bacterial resistance towards streptomycin. On its own, \(p\)-aminosalicylic acid lacked sufficient potency for routine clinical application. There were two other shortcomings. It was rapidly excreted via the kidneys, resulting in the need for
oral administration with quantities of 12g daily in four or more divided doses. This compounded the second problem, which was that it caused distressing gastrointestinal disturbance. The problem could not be overcome either by formulating it differently or by the synthesis of analogues. Once a range of alternative drugs became available in the 1980s, p-aminosalicylic acid was no longer prescribed.

**Cresols**

In 1886, Oswald Schmiedeberg claimed that cresol was not only more potent but also less toxic than phenol. As cresol consisted mainly of m-cresol, together with its ortho and para isomers, any reduced toxicity was probably due to the smaller amount that could dissolve in water. Kalle and Company of Frankfurt introduced chlorocresol as a bactericide in 1897. Many alkly, halo and haloalkylphenols have been introduced since then.

The findings of the first extensive investigation into their activity was reported in 1906 by Bechhold and Ehrlich, who found that although polyhalo compounds were more potent than monohalo compounds, they did not retain activity in the presence of serum. Mono-halophenols were subsequently shown by Laubenheimer to be less affected by the presence of serum. This was of considerable importance for compounds that were to be used in the clinic. Klarmann discovered that in higher molecular weight phenols the spectrum of antibacterial activity did not change uniformly with alteration to the chemical structure. In several instances he found that structural modification enhanced activity against most organisms, yet removed all activity against specific organisms. This phenomenon was to be encountered repeatedly in the antibiotic era.

A well-equipped unit supported by the Medical Research Council, the Rockefeller Foundation and the Bernhard Baron Trustees was opened at Queen Charlotte’s Maternity Hospital in London in 1931 with the objective of finding a solution to the problem of puerperal fever. This had been the cause of death in two or more out of every thousand women within days of giving birth. Leonard Colebrook, the bacteriologist in charge of the new unit, was particularly concerned about a form of the disease caused by haemolytic streptococci, in which there had been a mortality rate of over 25%. He collaborated with his cousin, a chemist who worked for the Reckitt company in Hull, in the development of a non-irritant antiseptic that could kill streptococci on the skin of the midwives’ hands. He experimented on himself by smearing his hands with virulent bacterial cultures, a procedure that led to the development of chloroxylenol solution as a non-irritant antistreptococcal hand disinfectant, which greatly reduced the incidence of puerperal fever caused by streptococcal infection. It remains the most important of the cresols to have been introduced into medicine.

**Hexachlorophene**

William Gump began an investigation of halogenated bisphenols in the laboratories of Givaudan-Delawanna in New York in 1937, which resulted in the development of hexachlorophene as a skin disinfecting and cleansing agent after the Second World War.
had ended. It had the advantage of retaining activity in the presence of soap and hence was introduced in creams, soaps and cleansing lotions sold to the general public.

Tragically, a manufacturing blunder in France led to the sale of a baby powder containing 6% hexachlorophene, which resulted in the death of 20 children before the cause was discovered. It was subsequently confirmed that severe neurotoxicity had previously occurred in infants after absorption of hexachlorophene through the skin on repeated application. This led the US Food and Drug Administration in 1972 to ban sales to the public of all formulations containing more than 0.1% hexachlorophene. Other drug authorities did likewise, with hexachlorophene being allowed to remain in use as a skin disinfectant for health care workers.

**HYPNOTICS**

Chloral hydrate was synthesised in 1832 by Justus Liebig, who also discovered that it decomposed into chloroform and formic acid when treated with alkali. This caught the attention of two investigators, Rudolf Buchheim and Oskar Liebreich, who both then discovered the hypnotic action of chloral hydrate.

Buchheim had wondered whether excessive alkalinity of the blood, which was thought to be a complicating factor in some diseases, could be reduced by administration of chloral hydrate. The idea behind this was that as treatment of chloral hydrate with caustic alkali liberated chloroform and formic acid, then alkaline blood should do likewise. Buchheim believed that the chloroform thus released in the blood might even be converted into hydrochloric acid, thereby supplementing the alkali-neutralising action of the formic acid. However, on taking a draught of chloral hydrate to test his hypothesis he and several of his colleagues quickly fell asleep. Buchheim thought this proved that chloroform had been released, but had not then broken down into hydrochloric acid. His investigation was abandoned and not reported until 1872, three years after Liebreich had introduced chloral hydrate as a hypnotic drug.

Liebreich, an assistant professor at Berlin University’s Pathological Institute, also tried to use chloral hydrate to liberate chloroform in the blood. Unlike Buchheim, he actually hoped the chloroform would induce unconsciousness. He was therefore delighted when experiments on rabbits confirmed his expectations. The animals awakened unharmed several hours later. When 1.35 g of chloral hydrate was administered to a disturbed individual by subcutaneous injection, he slept for 5 hours. A subsequent dose of 3.5 g in water kept him asleep for 16 hours. Liebreich published his findings in August 1869. Within a few months, chloral hydrate was in use all over the world as the first safe hypnotic, despite its unpleasant taste and the frequency with which it caused gastric irritation. An early shortage of supplies of chloral hydrate raised the price of a draught to three shillings and sixpence in the United Kingdom, or
just under US $1, leading to the expression, ‘A sleep costs a dollar!’ The shortage ended after Schering built a factory in Berlin to produce it. Daily consumption in both Britain and the United States passed the 1 ton mark within a decade, which no other contemporary drug even remotely rivaled. Chloral hydrate remains in use around the world.

![Image of trichloroethanol and triclofos](image)

It soon became evident that if any chloroform at all was released in the blood after administration of chloral hydrate, it could only be trace amounts. It is nowadays realised that the alkalinity of blood is so slight as to be unable to induce decomposition of chloral at all, but in the 1860s there was no awareness of the subtleties of Sørenson’s pH scale, which was not introduced until 1909. Joseph von Mering, a protégé of Schmiedeberg, correctly suggested that chloral hydrate was converted in the body into the active hypnotic trichloroethanol, but it was not until 1948 that there was experimental proof of this.

Trichloroethanol could not be administered as a drug because of its unpleasant taste, as well as a tendency to cause nausea. Its phosphate ester, triclofos sodium, was introduced by Glaxo in 1962. This is rapidly hydrolysed in the gut to liberate trichloroethanol. There was no problem with palatability when triclofos was formulated in an elixir as its water-soluble sodium salt.

The discovery of the hypnotic properties of chloral hydrate brought home to many people the potential of synthetic drugs as therapeutic agents. It also set the scene for what was to become for many years the sole alternative to basing the structures of synthetic drugs on those of natural products, namely the idea of designing a new drug that would decompose to release a pharmacologically active agent. An early example of this approach is seen when Schmiedeberg selected urethane as a potential anaesthetic in small animals. He thought it would break down in the body to release not only alcohol, a central nervous system depressant when large doses were consumed, but also ammonia and carbon dioxide, which were both known to be respiratory stimulants. The anaesthetic action of urethane that Schmiedeberg then observed was later shown to be due solely to the intact molecule, with neither carbon dioxide nor ammonia being released in the body. His hypothesis may have been wrong, but it resulted in the introduction of an anaesthetic that is still used in small animals.

![Image of urethane and chloralformamide](image)

The approach of designing drug molecules to liberate active substances can also be seen in analogues of chloral hydrate that were marketed in the 1880s. For example, Joseph von Mering patented chloralformamide as a hypnotic in 1889, more than 50 years after it had first been synthesised. He also believed that ammonia and carbon dioxide would be liberated as respiratory stimulants, which would counteract any respiratory depression that occurred as a result of overdosing. The new drug turned out to be no safer than chloral hydrate, though it was less irritating to the stomach.

**Sulfonmethane**

In the summer of 1887, Eugen Baumann at the University of Freiburg asked his colleague Alfred Kast to see whether some novel sulfur compounds that he had prepared had any
pharmacological activity. Kast began by injecting a suspension of 2 g of sulfonmethane into a
dog. Initially, there was no apparent reaction from the animal, but several hours later it
staggered and fell unconscious. The dog did not awaken until several hours later. The
experiment was repeated on other animals, confirming that sulfonmethane was a hypnotic. It
was marketed the following year by F. Bayer & Company.

As a hypnotic that combined palatability and absence of gastric irritancy with freedom from
circulatory disturbance, sulfonmethane was to be one of Bayer’s first profitable pharmaceu-
tical products. It retained its popularity until the introduction of the more rapidly acting
barbiturates rendered it obsolete.

The Barbiturates

Consideration of the chemical nature of the hypnotics discovered during the last two decades
of the nineteenth century convinced von Mering that a key feature in their molecular structure
was the presence of a carbon atom containing two ethyl groups. Knowing of work already
carried out by others on urethane and urea derivatives, he and Emil Fischer investigated
diethylacetyleurea, finding it to be as potent a hypnotic as sulfonmethane. Its bromo
derivative, carbromal, was later marketed by Bayer as a hypnotic.

After finding diethylacetyleurea to be a hypnotic, von Mering prepared 5,5-diethylbarbituric
acid, unaware that it had already been made 20 years earlier. The parent compound of this
series, barbituric acid, had been synthesised by von Baeyer in 1864, and is variously said to
have been so named after a young maiden with whom its discoverer was then in love, or, more
prosaically, on account of its first preparation being on St Barbara’s Day. After von Mering
established that 5,5-diethylbarbituric acid was a hypnotic in animals, he discussed the
compound with Fischer. The latter doubted the reliability of the synthesis and instructed his
nephew, Alfred Dilthey, to synthesise it and several related compounds. When tested on a dog,
5,5-diethylbarbituric acid proved to be the most potent of the 19 compounds that had been
synthesised and was much more potent than von Mering’s compound. This provoked Fischer
to remark that he now had the true compound, which explains why it was given the
proprietary name of Veronal (Latin: verus = true) when it was marketed by F. Bayer &
Company. Fischer filed a patent on the new hypnotic at the end of January 1903 and a
detailed report appeared the next year. All previous hypnotics, with the possible exception of
chloral hydrate, were now rendered obsolete.

When the United States entered the First World War in 1917, Congress passed the Trading
with the Enemy Act to allow American firms to manufacture unobtainable German drugs
covered by patents, such as Veronal® and Salvarsan®. Royalties were paid to the Alien
Property Custodian for distribution to the American subsidiaries of German companies when
the war ended. The Act required the American products to be given a new name approved by
the American Medical Association (AMA). This practice of giving a drug an approved, or
generic, name in addition to that chosen by its original manufacturer ultimately became
standardised throughout the world. In the case of Veronal\textsuperscript{8}, Roger Adams at the University
of Illinois devised a manufacturing process for the Chicago-based Abbott Laboratories. The
drug was then given the AMA approved name of barbital.

During the First World War, Chaim Weizmann (who later became the first president of
Israel) discovered that a bacterium known as \textit{Clostridium acetobutylicum} could convert cheap
starchy materials to acetone and \textit{n}-butanol. This was of immense military significance as the
United Kingdom was desperately short of acetone for the production of naval explosives.
Once peace was restored, the Weizmann process resulted in a sudden drop in the price of \textit{n}-
butanol, previously an expensive chemical. Carl Marvel and Roger Adams at the Urbana
campus of the University of Illinois synthesised \textit{5}-butyl-\textit{5}-ethylmalonic ester in 1920.\textsuperscript{67} This
was to be the key intermediate in the synthesis of the \textit{butyl} analogue of barbital, butobarbital
(also known as ‘butethal’), by Arthur Dox and Lester Yoder of Parke, Davis and Company.\textsuperscript{68}
This new barbiturate was about three times as potent as barbital, with a shorter duration of
action, which minimised any drowsiness on awakening. The increased potency and more rapid
metabolic destruction of butobarbital are both due to the enhanced lipophilicity caused by
introduction of the longer butyl group. This favours entry into the brain, which is the site of
action, and into the liver, which is the site of metabolic deactivation. Shonle and Moment of
the Eli Lilly Company in Indianapolis announced their synthesis of amylobarbital (now
known as ‘amobarbital’) a year after the introduction of butobarbital.\textsuperscript{69} Both drugs were of
similar potency, but the branched carbon atom on amobarbital rendered it more susceptible to
metabolic deactivation, shortening the duration of action still more. It and similar barbiturates
such as pentobarbitone and quinalbarbitone (secobarbitone) became highly popular hypnotics
until the 1960s, when mounting concern about both their habit-forming properties and use in
suicide led to their decline.

\textbf{Drugs Structurally Related to Barbiturates}

No hypnotics to challenge the barbiturates were developed until the 1950s, by which time there
was concern about accidental overdosing by drowsy patients, as well as their use in suicide
attempts. In 1952, Tagmann and his colleagues at Ciba in Basle announced that they had
found a potent hypnotic among a series of dioxotetrahydropyridines structurally related to the
barbiturates.\textsuperscript{70} The new compound, glutethimide, was initially hailed as safer than the
barbiturates – a claim that did not withstand the test of time.
Aminoglutethimide was marketed for the treatment of epilepsy in 1960 after Ciba researchers found it was a stronger anticonvulsant but had weaker sedative–hypnotic properties than glutethimide. In 1963, Ralph Cash, a paediatrician at the Sinai Hospital in Detroit, reported that it had induced the typical signs of Addison’s disease (adrenal insufficiency) in a young girl who had been receiving it for five months to control her epilepsy. After similar reports from other doctors appeared, laboratory studies revealed that the drug had blocked steroid biosynthesis. It was withdrawn from the market in 1966. Cash demonstrated that aminoglutethimide inhibited the desmolase enzyme that removed the side chain from cholesterol to form pregnenolone, a prerequisite for steroid hormone synthesis. Subsequently, it was administered to patients with Cushing’s disease in the hope that they might benefit from its ability to inhibit overproduction of corticosteroids, but results were disappointing. In the 1970s physicians began administering aminoglutethimide to women with metastatic breast cancer, supplementing the drug with dexamethasone to compensate for diminished cortisone levels in the body. The value of aminoglutethimide, especially in those women who had relapsed after initially responding to tamoxifen, is now established.

Another analogue of glutethimide was introduced by Chemie Grünenthal, a company established immediately after the Second World War by a soap and toiletries manufacturer keen to obtain a stake in the growing market for antibiotics, then in desperately short supply. Heinrich Mueckter, who qualified in medicine before the war, was appointed as research director on the basis of his wartime experience with the German army virus research group. In 1953, his assistant Wilhelm Kunz was given the task of preparing simple peptides required for antibiotic production. In the course of this he isolated a by-product that was recognised by a Chemie Grünenthal pharmacologist Herbert Keller to be a structural analogue of glutethimide. A series of related compounds were examined, from which one was examined in detail by Keller for its suitability as a hypnotic agent. Unusually, it did not abolish the righting reflex of animals – a standard laboratory test for hypnotic activity. Keller conducted a series of studies on the mobility of mice exposed to the drug, thalidomide, comparing it with several barbiturates and other central nervous system depressants. After investigating its toxicity in mice, rats, guinea pigs and rabbits, he came to the conclusion that it was a remarkably safe sedative.

Chemie Grünenthal approached manufacturers throughout the world, with the outcome that several who were keen to enter the market for sedative–hypnotics marketed it with their own brand name. This was to lead to the greatest tragedy in the history of modern drugs, for the new sedative was a teratogen.

Thalidomide was introduced on the German market in 1956. In November 1961, Hamburg paediatrician Widukind Lenz reported a large increase in the number of infants with phocomelia attending ten clinics in North Germany. Instead of limbs, they had stumps. This had previously been one of the rarest malformations known, with no cases having been seen in these clinics in the decade prior to 1959. Yet there were 477 cases in 1961. Lenz attributed the increase to the taking of thalidomide by mothers during the first trimester of their pregnancies and notified Chemie Grünenthal and the authorities. The report of thalidomide teratogenicity was immediately picked up and publicised by a German newspaper, forcing the manufacturer to withdraw the drug. Like most others introduced up till then, it had never been tested for teratogenicity.
By the time thalidomide was withdrawn, 3000 deformed babies had already been born in Germany and at least twice that number elsewhere. The United States was spared because Frances Kelsey at the Food and Drug Administration had not approved a new drug application, having been dissatisfied with the limited safety data that had been submitted. At that time, it was only the United States that required manufacturers to seek government approval before launching a new drug. Within a few years of the thalidomide disaster, countries around the world had quickly emulated the American system.

Barbiturate Anaesthetics

Intravenous medication did not become feasible until after the development of the hypodermic syringe by Alexander Wood of Edinburgh in 1853. The earliest attempt at intravenous anaesthesia was due to the work of Pierre Oré of Bordeaux, who reported to the Surgical Society of Paris in 1872 that he had injected a solution of chloral hydrate and achieved deep enough anaesthesia to remove a fingernail. Oré published a detailed report of a further 36 operations in which he had used the technique with some success, but in one case the patient died. It was not until 1905 that further development occurred when N.P. Krawkow of St Petersburg successfully administered a saline solution containing the Bayer Company’s recently introduced urethane analogue Hedonal. Fedoroff subsequently used this method in more than 500 operations. The technique was taken up in Russia and some parts of Europe, where it stimulated others to seek more suitable drugs.

Daniel Bardet reported in 1921 that he had anaesthetised patients with injections of Somnifen, a water-soluble formulation of barbital and allobarbital. He found that recovery was too slow, and patients awoke with headaches. Amobarbital, butallylonal and pentobarbital were occasionally used in the latter half of the decade. Particularly disconcerting, however, was a tendency for anaesthesia to deepen alarmingly without warning. This was because the delay in its onset prevented the anaesthetist from knowing how much drug was required to render the patient unconscious. Not until I.G. Farben introduced hexobarbital in 1931 did a safe intravenous anaesthetic become available. With it, the onset of action was rapid and so the anaesthetist could control the level of anaesthesia by giving the injection slowly.

Hexobarbital was synthesised by the chemists Kropp and Traub at Elberfeld. Its rapid onset of anaesthesia was due to the replacement of a hydrogen atom on one of the barbiturate ring nitrogen atoms by a methyl group. This rendered the molecule less water soluble and more lipophilic. Small though this molecular change may have been, it ensured rapid transposition of the drug from the blood into the brain cells. As a consequence, patients fell unconscious in the few seconds it took for the blood to carry the anaesthetic to the brain from the site of injection. Hexobarbital was deservedly successful, and it is estimated that over the next 12 years some 10 million injections of it were administered.

Even before the first reports of the success of hexobarbital had begun to circulate, Donalee Tabern and Ernest Volwiler of Abbott Laboratories were on the trail of the drug that was ultimately to render it obsolete. Their work on pentobarbital encouraged them to seek very short-acting barbiturates, probably with a view to introducing them as hypnotics free from any tendency to produce a hangover. They followed up old reports stating that
sulfur-containing thiobarbiturates were chemically less stable than the familiar oxobarbiturates. Thiobarbiturates had been among the earliest barbiturates examined in 1903 by Fischer and von Mering, but had been rejected after an oral dose of the sulfur analogue of barbital had killed a dog. Notwithstanding, Tabern and Volwiler pursued their idea that a chemically unstable thiobarbiturate might decompose fast enough in the body to ensure that its effects quickly wore off. By 1934 they were convinced that thiopental, the sulfur analogue of pentobarbital, was a promising agent. Doubtlessly inspired by the recent success of hexobarbital, they arranged for thiopental to be investigated by Ralph Waters, who had just completed his pioneering investigations into cyclopropane anaesthesia, at the University of Wisconsin Medical School, Madison, and by John Lundy of the Mayo Clinic in Rochester, Minnesota. Both confirmed the superiority of thiopental over existing intravenous anaesthetics. It ultimately achieved recognition as the single most useful agent for the induction of anaesthesia prior to the administration of an inhalational anaesthetic. It was only in the 1990s that it was rivalled by propofol. It also became widely used as an intravenous anaesthetic for short operations.

**Anticonvulsants**

Phenobarbital was one of the compounds reported by Fischer and Dilthey in their paper of 1904. It was later found to be superior to barbital and was marketed by F. Bayer & Company under the proprietary name of Luminal. For half a century it was a commonly prescribed hypnotic and sedative, but it remains in use today principally on account of its anticonvulsant activity. This was discovered by chance shortly after its introduction into the clinic, when a young doctor, Alfred Hauptmann, supplied it to epileptic patients in his ward who kept awakening him at night due to their fits. He expected the hypnotic to keep them asleep during the night, but did not expect that the incidence of their fits would decline during the day, particularly in those with grand mal epilepsy. At first, there were few who believed Hauptmann’s claims that he had stumbled upon the first truly anti-epileptic drug that did not produce the severe sedation hitherto associated with the use of bromides. Only after the First World War was there general recognition of this valuable property of phenobarbital.

![Phenobarbital and Phenytoin](image)

Tracy Putnam, the Director of the Neurological Unit of the Boston City Hospital, initiated experiments in 1934 aimed at finding a less sedating anticonvulsant than phenobarbital. He and Frederick Gibbs established the first electroencephalographic laboratory in the world designed for routine clinical studies of brain waves. An important observation to emerge from the new laboratory was that epileptic seizures were accompanied by an electrical ‘storm’ in the brain. This led Putnam to conclude that it might be possible to induce convulsions in laboratory animals by applying an electrical current to the brain. Furthermore, it might also be possible to quantify the strength of current required, thereby affording a method of recognising whether a drug was able to give some degree of protection to the animal. Having then set up an improvised piece of apparatus, Putnam and Gibbs demonstrated that phenobarbital markedly raised the convulsive threshold in cats.

Putnam next sought a wide variety of phenyl compounds from several chemical manufacturers, believing that the phenyl group in phenobarbital was somehow responsible...
for its efficacy. Only Parke, Davis and Company responded. They provided 19 analogues of phenobarbital, all of which had been found to be inactive as hypnotics. Putnam screened these, as well as over a hundred other available chemicals. A few were active but, with one exception, were too toxic for clinical use. The exception was one of the Parke, Davis and Company compounds, phenytoin, which was more effective in protecting cats from electrically induced convulsions than even phenobarbital. As it was known to have no hypnotic or other untoward effects, this seemed to be just what Putnam had been seeking.

Putnam gave phenytoin to Houston Merritt for clinical evaluation in 1936. The first patient to receive the drug had suffered from seizures every day for many years, but as soon as his treatment began these ceased permanently. Subsequent studies confirmed that phenytoin was at least as effective as phenobarbital, with the added advantage of causing less sedation.\(^87\) Paradoxically, this absence of marked sedation initially prejudiced many physicians against accepting the new drug!

After the Second World War ended, Parke, Davis and Company initiated a major research project to find a less toxic drug to replace Abbott’s troxidone in petit mal. This involved the synthesis and testing of over 1000 aliphatic and heterocyclic amides. This resulted in the discovery of three useful anticonvulsants, namely phensuximide\(^88\), methsuximide\(^88\) and ethosuximide. The last of these was originally synthesised in 1927\(^89\) and was put on the market in 1958. It remains in use for the treatment of petit mal absence seizures in children.

ICI scientists also tried to find improved anticonvulsants in the early 1950s. Herbert Carrington at the company’s research laboratories in Manchester considered that the new hydantoins being developed by Parke, Davis and Company, although relatively free of sedating properties, produced too many side effects. Barbiturates, in contrast, were usually free from these, but instead caused sedation. However, as not all sedating barbiturates were anticonvulsants, Carrington concluded that it should be possible to find a barbiturate analogue in which this separation of activity was reversed. This led on to the development of primidone by Charles Vasey and William Booth.\(^90\) It was given its first clinical trial in 1952 and results were satisfactory in grand mal epilepsy.\(^91\) How much of its efficacy is due to phenobarbital formed from it and how much is due to unchanged primidone is uncertain, but primidone is prescribed when neither phenytoin nor carbamazepine is acceptable.

**IMIDAZOLINES**

Piperazine was synthesised at the University of Breslau in 1888 by Alfred Ladenburg.\(^92\) When he discovered that it formed a soluble salt with uric acid, he suggested that this might dissolve the deposits of uric acid that caused much pain in patients with gout.\(^93\) Piperazine was immediately marketed for this purpose and remained in use well into the twentieth century.
Despite repeated criticism on the grounds that it was ineffective, the same situation arose with 2-methylimidazoline, which Ladenburg synthesised in 1894. After clinical studies were conducted at his suggestion, it was marketed for the treatment of gout on the grounds that it dissolved uric acid.

In 1935, Henry Chitwood and Emmet Reid at the Chemistry Department in Johns Hopkins University decided to reinvestigate 2-methylimidazoline and its homologues. Only the methyl homologue had any effect on the acidity of the urine, a pointer to increased excretion of uric acid — which was the usual mode of action of drugs that relieved gout. Toxicity decreased as the methyl group was replaced with longer alkyl groups. As this was the reverse of the tendency usually found in a series of homologues, it prompted researchers at the Ciba laboratories in Basle to re-examine the series of compounds. They obtained the opposite effect so far as the influence of chain length on toxicity was concerned, contradicting the earlier claims. In the course of this investigation of the toxicity of imidazolines, a drop in blood pressure caused by dilation of peripheral blood vessels was observed. When Ciba chemists introduced cyclic substituents such as benzene and naphthalene rings at the 2-position of the imidazoline ring, the toxicity decreased. The most potent of these compounds, tolazoline, was found to have weak adrenergic blocking activity and was introduced clinically. Its use had to be limited to the treatment of Raynaud’s disease and certain spastic vascular disorders since it stimulated the heart. Unexpectedly, the naphthyl analogue increased blood pressure by acting as a vasoconstrictor. It was introduced into clinical practice in the early 1940s as a long-acting nasal decongestant called ‘naphazoline’.

In an expansion of their studies on imidazolines, Ciba researchers investigated other heterocyclic compounds containing two nitrogen atoms. A series of phthalazines were found to be active in screens for hypotensive activity, from which hydralazine emerged as a long-acting peripheral dilator. It became the first orally active peripheral vasodilator to be introduced for the treatment of high blood pressure. With regular use, patients experienced side effects and became tolerant to it. As a result, other drugs superseded it. However, hydralazine became popular once again when it was found that tolerance was due to physiological compensatory mechanisms that could be overcome by combining it with a beta-blocker and a diuretic. Since the dose of hydralazine required in such a combination was smaller than that when used on its own, patients experienced fewer side effects. It continues to be widely used.

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Drugs Originating from the Screening of Dyes

In 1777, Wilhelm Friedrich von Gleichen-Russworm described how he had stained microbes with the natural dyes indigo and carmine so that they could be examined under the microscope. His procedure was refined in 1869 when the botanist Hermann Hoffmann replaced indigo with the synthetic dye magenta, which had first been marketed ten years earlier. Carl Weigert at the University of Breslau then adapted the method for use in pathological investigations, employing a microtome to slice infected tissues so thinly that they could be stained and viewed under a microscope. He also selectively coloured different cellular structures in tissue slices with synthetic dyes. This caught the imagination of his younger cousin, Paul Ehrlich.

Ehrlich entered the University of Breslau in 1872, where Weigert persuaded him to study medicine. When Wilhelm Waldeyer moved from Breslau to the new University of Strassburg, Ehrlich went with him. As his tutor, the great anatomist had both welcomed Ehrlich into his home and encouraged him to continue with his microscopic investigations. Stimulated by reading Huebel’s book on lead poisoning, which explained that chemical analysis of various organs had established that lead concentrated in the brain, Ehrlich examined slides of brain tissue in the hope of determining where the lead was stored. When this proved to be a futile exercise, it forced him to change his approach. Instead of lead salts, he injected dyes that could easily be detected in the cellular components where they concentrated.

In 1874, having completed his pre-clinical studies, Ehrlich returned to Breslau, where he avoided all unnecessary clinical involvement. He preferred to study in the laboratory of Julius Cohnheim, who encouraged him, Weigert and Robert Koch in their endeavours. The final part of Ehrlich’s medical studies was completed at Leipzig. His doctoral dissertation, submitted in 1878, was entitled *Beiträge zur Theorie und Praxis der Histologischen Färbung* (Contributions to the Theory and Practice of Histological Staining). It was highly critical of histologists for failing to base their work on a theoretical understanding of how dyes bind to tissue components.

On taking up his first post at the Charité Hospital in Berlin, Ehrlich spent most of his time on histological studies, especially in the field of haematology. He continued to strive for an understanding of the factors influencing the uptake of dyes by cells, and came to the conclusion that the size of the molecule was critical. He soon became disillusioned with the limitations imposed by examination of tissues exposed to dyes only after the death of the animal from which they were taken. By 1885, he had developed a new approach in which he injected dyes into living animals, then left them to diffuse into the tissues before killing them. For the first time, it became possible to examine the disposition of chemical substances in living animals, a process that Ehrlich called ‘vital staining’. The wide diversity of synthetic dyes that were available enabled Ehrlich to draw conclusions about the influence of chemical structure on the distribution in live animals of different types of coloured molecules. These conclusions still influence drug design. For example, he observed that acidic dyes possessing the sulfonic acid function introduced by dye manufacturers to enhance water solubility were...
unable to penetrate into the brain or adipose (fat) tissue. In marked contrast, basic dyes such as methylene blue and neutral red readily stained these tissues. To explain this, Ehrlich drew an analogy between the extraction of fat-soluble, basic alkaloids from alkaline solution into ether and the transfer of basic dyes from the alkaline blood to the brain. Alkaloids could not be extracted into ether from acid solutions, where they existed in the form of their salts, which were insoluble in ether, just as acidic dyes existed as water-soluble salts in blood. This remarkably accurate assessment of the situation was made during the years 1886 and 1887.

In 1881 Ehrlich stained bacteria with methylene blue, which had been synthesised by Heinrich Caro five years earlier. Four years later, he found that this lipophilic dye had a strong affinity for nerve fibres, leaving other tissues unaffected. He described it as being neurotropic, for upon injecting it into a living frog all the nerve fibres were gradually tinted blue. Ehrlich then reasoned that as methylene blue stained nerves it might possibly interfere with nervous transmission and exert an analgesic action. In 1888, he and Arthur Leppmann gave it to patients suffering from a variety of severe neuritic and arthritic conditions. They found that it did relieve pain, but its tendency to damage the kidney discouraged its use as an analgesic. Examination of Ehrlich’s unpublished letters by Henry Dale revealed that he wrote to chemists in the dyestuffs industry asking advice about the possibility of obtaining analogues of methylene blue that might be more potent analgesics.

In 1891, Ehrlich carried out further experiments with methylene blue after returning from Egypt, where he had gone to recuperate from tuberculosis. Knowing that it stained the plasmodia that caused malaria and that it could be administered to patients, he and Paul Guttmann administered daily five capsules each containing 100 mg of methylene blue to two patients who had been admitted to the Moabit Hospital in Berlin with malaria. Both recovered as a result of this treatment. Although methylene blue was later found to be ineffective against the more severe manifestations of the disease experienced in the tropics, this cure of a mild form of malaria represented the first instance of a synthetic drug being used with success against a specific disease. In 1995, it was reported that methylene blue had exhibited high antimalarial activity in laboratory studies.

Ehrlich could not pursue his work with methylene blue any further, for two reasons. Firstly, the inability to infect animals with malaria prevented testing of potential drugs in the laboratory, a prerequisite for the development of any chemotherapeutic agent for use in human or veterinary medicine. Secondly, he was working in Robert Koch’s Institute for Infectious Diseases in Berlin, where his skills were fully deployed in transforming Emil von Behring’s diphtheria antitoxin into a clinically effective preparation. This was later to result in his sharing the Nobel Prize for Medicine with Mechnikov in 1908.

MEDICINAL DYES

Following his early investigation of the action of the organic arsenical Atoxyl against cultures of trypanosomes (see Chapter 7), Paul Ehrlich examined more than 100 synthetic dyes that were injected into mice infected with either Trypanosoma equinum, the organism that caused mal de Caderas in horses, or T. brucei, which caused nagana in cattle. The only dye to exhibit activity belonged to the benzopurpurin series. Ehrlich named it ‘Nagana Red’. Like arsenious acid, it caused the disappearance of trypanosomes from the blood of the mice for a short time, the mice surviving five or six days instead of the usual three or four.
Ehrlich believed that the low solubility of Nagana Red was the cause for it being poorly absorbed into the circulation from the site of its injection under the skin. He therefore contacted the Cassella Dye Works near Frankfurt, the longest established of several dye manufacturers in the area (it was soon to become affiliated with the larger Hoechst Dyeworks). Ehrlich asked for a derivative of the Nagana Red with an extra sulfonic acid function to enhance water solubility. Ludwig Benda was able to give him a dye first prepared in 1889, which was subsequently to be called ‘Trypan Red’. Injections of it cured mice infected with \( T. \textit{equinum} \) but not with other strains of trypanosomes.\(^7\) The British Sleeping Sickness Commission requested supplies of the dye for trials in Uganda, but the results were disappointing since doses high enough to be effective against the disease were found to be likely to cause blindness and sometimes death.

Ehrlich obtained about a further 50 derivatives of Trypan Red from the Cassella Dye Works. The most active of these were more potent than Trypan Red itself. A supply of the 7-amino derivative of Trypan Red was tested in Africa during an expedition led by Koch in 1906, but it proved to be no better than the parent compound.

**SURAMIN**

Maurice Nicolle and Felix Mesnil at the Pasteur Institute examined analogues of Trypan Red supplied by Friedrich Bayer & Company, the leading manufacturer of acidic azo dyes. The French workers disclosed that a single injection of Trypan Blue could cause the disappearance of all trypanosomes from the blood.\(^8\) Further trials showed that it had a mild action against several different strains of trypanosomes that caused disease in cattle, being more effective than either Atoxyl\(^9\) or Trypan Red but still not acceptable for human use. After a demonstration that it could cure piroplasmosis (babesia), it was introduced into veterinary practice.\(^9\)

Wilhelm Roehl moved from Ehrlich’s laboratory in Frankfurt, where he had been testing the azo dyes provided by the Cassella Company, to join the Bayer research group at Elberfeld in 1905. He found that none of the dyes prepared by the company for Nicolle and Mesnil were effective in his own infected mice. Nevertheless, Bernhard Heymann, who was director of the scientific laboratory at Elberfeld, asked Oskar Dressel and Richard Kothe to synthesise new analogues. Roehl requested that colourless compounds should be made since a drug that tinted the skin would not be acceptable to patients. Dressel and Kothe therefore synthesised analogues of one of the least coloured Bayer dyes that had been screened by Nicolle and Mesnil, namely Afridol Violet. Although this had only feeble activity, several red analogues
exhibited stronger activity against trypanosomes. It was this advance that led to priority being
given to the project in 1913. Superior analogues were developed, with the first patents being
applied for just before the outbreak of the First World War. At this point there were
arguments within the company over the wisdom of continuing with a line of research that had
not delivered a truly outstanding drug. Only the insistence of Heymann prevented the project
from being dropped. By the autumn of 1917, more than 1000 naphthalene ureas had been
synthesised and tested. Only then did the long-sought agent emerge in the form of a colourless
compound that had remarkable antitrypanosomal activity both in experimental animals and
in humans. It had the code name of Bayer 205 and was later marketed by Bayer under the
proprietary brand name of Germanin®. Its chemical structure was not revealed because F.
Bayer & Company feared this would enable foreign manufacturers to develop similar
products.10

The first reports of the discovery of the new trypanocide began to circulate outside the
Elberfeld laboratories towards the end of 1920. For a while the drug was only made available
to German doctors and a few foreign investigators who undertook not to allow it to pass into
the hands of anyone capable of determining its chemical structure. This sorely irked Ernest
Fourneau, head of the medicinal chemistry laboratory at the Pasteur Institute. Originally
trained in France as a pharmacist, he had then studied under leading German chemists,
including Emil Fischer and Richard Willstätter. On returning to France, he had been anxious
to lessen the dependence of his native country on drugs imported from Germany. He was now
determined to establish the chemical structure of Bayer 205 by one means or another. To this
end, he conducted a critical examination of 17 Bayer patents covering trypanocidal ureas
derived from naphthalene sulfonic acids. His persistence enabled him to conclude that Bayer
205 must have been one of 25 possible structures. He then synthesised several of these and had
them tested on infected mice. After one exhibited antitrypanosomal properties identical to
those of Bayer 205, Fourneau published its structure in 1924, calling it Fourneau 309.11,12
Because the structure had never been previously published, F. Bayer & Company could not
claim that its patents were being infringed. For this reason, disclosure of structures thereafter
became standard practice in pharmaceutical patents. It was not until 1928 that F. Bayer &
Company finally admitted that Bayer 205 was identical to Fourneau 309. The approved name
of the drug is suramin.
The success of suramin can be measured by the fact that three-quarters of a century after its discovery it remains one of the principal drugs for the prevention and treatment of trypanosomiasis. In keeping with Ehrlich’s observation concerning dyes featuring the sulfonic acid function, it cannot enter the central nervous system, ruling out its use in advanced forms of sleeping sickness. However, of even more significance than its undoubted therapeutic value was the stimulus suramin gave to the subsequent development of chemotherapy in Germany. Within 12 years of its discovery, researchers at Elberfeld had developed the first effective synthetic antimalarials, the sulfonamides, and several other chemotherapeutic agents by an extension of the approach that had led to the introduction of suramin.

**PAMAQUIN**

Charles Louis Alphonse Laveran isolated the protozoal parasite that caused malaria as long ago as 1881, but it was not until 1924 that Wilhelm Roehl devised a technique for screening potential antimalarial drugs by administering them to canaries in the Bayer laboratories at Elberfeld. Compounds that appeared promising in the new screening programme were subsequently tested in syphilitic patients suffering from general paralysis of the insane who had been therapeutically inoculated with malarial parasites to produce fever. This approach had recently been introduced in Vienna by Julius Wagner-Jauregg, who claimed that at least one-third of his paralysed patients recovered after such treatment.

Once it had become possible to screen potential antimalarials, Werner Schulemann and his colleagues at Elberfeld, Fritz Schönhofer and August Wingler, followed up the earlier suggestion by Paul Ehrlich that derivatives of methylene blue should be synthesised as potential antimalarials. They began by substituting a diethylaminoethyl side chain on one of the methyl groups in the blue dye. Roehl found this effective in canaries, with a therapeutic index of 8 (i.e. the ratio of the toxic dose to the therapeutic dose), but he was concerned that as the new compound was a strongly coloured dye there could be consumer resistance to its use. To avoid this problem, Schulemann switched his attention to quinolines, but he retained the basic side chain of the active methylene blue analogue as he was convinced that it was essential for antimalarial activity. After it was substituted on to 8-aminoquinoline, the resulting compound cured infected canaries. This quinoline subsequently served as the lead compound from which a diverse range of analogues were then synthesised and tested on canaries. The initial strategy was to examine the effect of altering the point of attachment of the side chain to the quinoline ring and then to investigate what happened when the side chain was varied in just about every conceivable manner. In order to increase the similarity to quinine, a methoxyl group was placed on the 6-position of the quinoline ring. As if all this were not enough, Schulemann and his colleagues in addition then introduced a variety of heterocyclic ring systems other than quinoline. Literally hundreds, possibly even thousands, of compounds were prepared and tested by the small group of researchers at Elberfeld. In 1925, a promising compound with a therapeutic index of 30 was selected for clinical evaluation. It was initially tried in patients who had been infected with the malarial parasite as part of the Wagner-Jauregg regimen. The new drug was effective, and Roehl confirmed that it was also able to cure patients with naturally acquired malaria. Clinical trials throughout the world followed, and then the drug was marketed as Plasmoquine. It was given the approved name ‘pamaquin’, though its chemical structure was not disclosed until 1928, after the company had changed its policy on such matters.
The life cycle of the malarial parasites (sporozoites) after they enter the blood of a human bitten by a female anopheline mosquito is complex. Within an hour, liver cells are invaded and the sporozoites begin to divide, ultimately causing the cells to rupture. This releases merozoites into the blood, which then penetrate the red cells to initiate the erythrocytic phase of the disease. The merozoites multiply until the red cells rupture, causing the patient to experience chills, fever and sweating. The released merozoites then attack other blood cells to renew the cycle, accounting for the periodicity of malarial attacks. Quinine suppresses this erythrocytic stage of the disease. Roehl, however, discovered that the action of pamaquin was quite different, and it was clearly not simply a substitute for quinine. Large doses of pamaquin lowered the incidence of relapses among patients with benign tertian malaria caused by *Plasmodium vivax*. This is the commonest type of malaria and is so named as fewer deaths occur than with malignant tertian malaria, in which patients are infected with *P. falciparum*. Outright cures were even reported among those who could tolerate its inevitable side effects. However, better results were obtained when small doses of pamaquin were administered in conjunction with quinine. It was only some years later that it was established that pamaquin acted by destroying parasites that persisted in the liver. These were responsible for the characteristic relapsing fever associated particularly with *P. vivax* infection. The combination of pamaquin with quinine eradicated the infection in both the liver and the blood, thus producing outright cures.

Following the clinical introduction of pamaquin, the Joint Chemotherapy Committee of the Medical Research Council and the Department of Scientific and Industrial Research sponsored an ambitious programme of research in British universities, with the aim of developing new antimalarial drugs. In 1929, the first of many publications came jointly from Robert Robinson at University College in London and George Barger at the University of Edinburgh.15 This described how they had been able to establish the chemical nature of pamaquin before its structure had been disclosed by what was by then no longer Bayer but I.G. Farbenindustrie. They then proceeded to make analogues of it. Although nothing superior to pamaquin arose out of either this collaboration or similar investigations by Fourneau in France, Magidson in Russia, Hegener, Shaw and Manwell in the United States or Brahmachari in India, the scene was set for a massive wartime effort that followed soon after. The I.G. Farbenindustrie group at Elberfeld, however, did succeed in discovering more effective quinoline antimalarials in the 1930s, showing how far ahead they were of their international competitors.

**Mepacrine (Quinacrine)**

The dichloro analogue of the triphenylmethane dye magenta was synthesised in 1909. Paul Ehrlich discovered not only that it had weak trypanocidal activity but also that this was due to its contamination by small amounts of acridines. He then asked Louis Benda at Farbwerke vorm. Meister, Lucius und Brüning to synthesise the yellow acridine dye, which was to become known as trypaflavine after Ehrlich found it to be the most potent trypanocide with which he had ever worked.16 It was highly effective against virulent strains of *Trypanosoma brucei* in mice, yet proved to be worthless in larger animals or humans. However, Ehrlich’s assistant Kiyoshi Shiga reported that, like magenta, it had bactericidal properties.17 This was confirmed by Carl Browning, a former associate of Ehrlich who had returned to the University of Glasgow Medical School, where he tested a wide variety of dyes for antibacterial activity.18 He discovered that trypaflavine was even effective against pathogenic organisms in the presence of serum. After war broke out the following year, the newly established UK Medical Research Committee asked Browning to continue his work on trypaflavine with a view to finding an antiseptic that could be used on deep wounds. Browning set up a laboratory in the Bland-Sutton Institute of Pathology at the Middlesex Hospital, London, where two dyes were
selected for trial in casualty clearing stations at the front lines and in base hospitals.\textsuperscript{19} These were brilliant green and trypaflavine, the latter now being renamed as acriflavine since it had no clinical value in trypanosomiasis. It was not until 1934 that it was realised that acriflavine was a mixture of two components, one of which was introduced during the Second World War under the approved name of proflavine.\textsuperscript{20} Aminacrine, a non-staining analogue of acriflavine, was also made available at that time.

By 1926 Robert Schnitzer of I. G. Farbenindustrie was able to control acute streptococcal infections in mice by administering, either orally or by injection, large doses of 9-aminoacridines substituted with a nitro group at the 3-position.\textsuperscript{21} One of these nitroacridines incorporated the same basic side chain that had conferred antimalarial activity on pamaquin. While effective against both trypanosomes and streptococci in animals, this particular nitroacridine was not potent enough to be considered for clinical use. An analogue of it prepared as a potential trypanocide by Schnitzer and Silberstein was shown to be more effective as an antibacterial agent. It was marketed as Entozon\textsuperscript{®} (also known as Nitroakridin 3582), but it caused severe tissue irritation at the site of injection, as well as unpleasant side effects. A closely related compound in which the nitro group was replaced with a chlorine atom was the most promising among more than 12000 compounds synthesised at Elberfeld that were entered in a screening programme that Walter Kikuth had established to discover a new antimalarial. The superiority of the chloroacridine was probably recognised in 1930, though the first report appeared in 1932.\textsuperscript{22} It was initially called Plasmoquine E\textsuperscript{®}, but to avoid confusion with pamaquin this was changed to Erion\textsuperscript{®} and then to Atebrin\textsuperscript{®}. It was later given the approved name of mepacrine; it is also known as quinacrine.

Kikuth’s original tests on mepacrine had convinced him that its action was similar to quinine insofar as, unlike pamaquin, it could kill merozoites in the erythrocytic phase of malaria. This meant that it could suppress the symptoms of malaria and cure those types of malaria in which the parasites did not persist in the liver cells. Although it was marketed throughout the world as a substitute for quinine, its full potential was not recognised until after the outbreak of the Second World War.

As the war clouds gathered, the importance of a quinine substitute was recognised since Germany had difficulty obtaining supplies of quinine during the First World War. Now the tables were likely to be turned if the Japanese took control of the East Indies, from whence came most of the world’s supplies of cinchona bark. Both pamaquin and mepacrine were
included in the Association of British Chemical Manufacturers’ list of essential drugs that would need to be produced in the United Kingdom if war broke out. ICI were requested to devise suitable manufacturing processes, and by September 1939 mepacrine was being manufactured in a pilot plant. Shortly afterwards, full-scale production to meet the requirements of the armed forces had begun. In 1941, the American government also responded to the threat of war. One of its earliest moves involved the Winthrop Chemical Company, which had been set up after the First World War to distribute Bayer Pharmaceuticals in the United States following the purchase from the Custodian of Enemy Property, by Sterling Drug Inc., of the Bayer Company of New York. By a subsequent agreement concluded in 1926, the newly constituted I.G. Farbenindustrie, which had taken over control of Bayer in Germany, became half-owners of Winthrop. Three months before the bombing of Pearl Harbor brought the United States into the war, a government antitrust suit severed the ties between the Winthrop Chemical Company and I.G. Farbenindustrie, leaving it a wholly American owned company. When the Japanese moved into the East Indies, Winthrop was called upon to supply mepacrine for the US armed forces. Prior to this, the company had merely produced about 5 million of its Atabrine® brand tablets annually from six chemical intermediates imported from Germany. Winthrop responded by sublicensing 11 leading American manufacturers on a royalty-free basis, and the outcome was that in 1944 alone some 3500 million mepacrine tablets were produced in the United States. This and the wartime effort to produce large amounts of penicillin laid the foundations for the United States to become the biggest producer of pharmaceuticals in the post-war world.

When British and American production of mepacrine first began, the drug was considered to be nothing more than a synthetic substitute for quinine. As a result of its widespread use by American forces in the Far East, it became apparent that mepacrine was superior to quinine for the treatment and suppression of malaria. While the war lasted, great care was taken to keep this vital information from the enemy so that they would continue to use quinine, an inferior drug.

The chance observation that the condition of a patient suffering from the chronic autoimmune disease of the connective tissues known as lupus erythematosus improved dramatically while taking mepacrine led to a detailed study that confirmed its value in lupus. Furthermore, this clinical trial also revealed that in patients with associated rheumatoid arthritis this also ameliorated as their skin condition improved. This led to further trials not only of mepacrine but also of other antimalarial drugs in patients with rheumatoid arthritis. Eventually the value of chloroquine and hydroxychloroquine for treating rheumatoid arthritis was generally recognised.

**Chloroquine**

During their North African campaign, German troops were equipped with supplies of sontoquine. This was a quinoline compound that could be considered as an analogue of mepacrine in which one of the benzene rings (i.e. that containing the methoxyl group) was absent. Samples of sontoquine were obtained from captured German prisoners of war and then sent to the United States for analysis. Particular attention was paid to the fact that sontoquine was an aminoquinoline substituted in the 4-position rather than the 8-position. Biological screening of a close analogue in which the methyl group on the 3-position of sontoquine was absent revealed outstanding antimalarial activity. This analogue, chloroquine, had been synthesised by Hans Andersag at Elberfeld in 1934 at the same time as sontoquine, and a German patent on it was awarded to the I.G. Farbenindustrie. Wilhelm Roehl’s successor, Walter Kikuth, had dismissed it as being toxic, preferring sontoquine instead. The American investigators, however, found chloroquine to have fewer side effects than mepacrine, as well as reducing malarial fevers more quickly. Another important advantage over mepacrine was that it did not colour the skin yellow. It was not possible to institute large-
scale production of chloroquine before the war ended, but eventually it rendered mepacrine obsolete.

Resistance of most strains of *Plasmodium falciparum* towards chloroquine has developed during the years since the introduction of this drug. In benign tertian malaria caused by *P. vivax*, *P. ovale* or *P. malariae*, chloroquine can be an effective treatment. It is also widely used in the prophylaxis of malaria in many regions throughout the world, often in combination with another antimalarial agent.

**Primaquine**

In the United States, the government Office of Scientific Research and Development established a research programme to discover antimalarial drugs. From 1941 to 1945, over 14,000 compounds were screened, around one-third of these being new substances. Robert Elderfield and his colleagues at Columbia University in New York contributed to the programme by noting that among the vast range of substituted 8-aminoquinolines related to pamaquin, few primary or secondary amines had been reported. A variety of such compounds were then synthesised, resulting in the emergence of primaquine as a less-toxic analogue of pamaquin, which it immediately superseded as the drug of choice for eradication of benign tertian malaria. No superior agent for this purpose has been found.

**Impact of Research on Antimalarials**

The research that was involved in developing synthetic antimalarial drugs from the mid-1920s to the mid-1940s in Germany and in the United States and the United Kingdom during the Second World War completely transformed the process of drug discovery from something that could readily be conducted in academic institutions or small commercial laboratories into a much more highly involved endeavour involving the preparation of large numbers of synthetic drugs by teams of chemists who received early information on their biological properties that could then be utilised in the selection of the next round of compounds to be prepared. The foundations for drug research in the second half of the twentieth century were firmly established by the end of the Second World War. The rate of discovery of new drugs since then has been unparalleled in any other period of history.

**THE ANTIBACTERIAL SULFONAMIDES**

At the University of Breslau in 1913, Philipp Eisenberg found that the azo dye known as chrysoidine possessed powerful antiseptic properties. Twenty years later, phenazopyridine was introduced as a urinary antiseptic by Ivan Ostromislensky, a New York industrial chemist who had suspected it might have bacteriostatic activity. After confirming this to be the case,
he noticed that it imparted a red colour to the urine of animals. This observation was
profitably exploited by marketing it as a relief for urinary tract pain. It is still prescribed even
though it has questionable efficacy as a urinary antiseptic.

Sulfamidochrysoidine (Prontosil Rubrum®)

In 1927, I.G. Farbenindustrie opened an outstandingly well-equipped suite of new research
laboratories at Elberfeld. Gerhard Domagk was appointed as Director of the Institute of
Experimental Pathology, with the responsibility of continuing the search initiated by Robert
Schnitzer at Hoechst for a drug effective against generalised bacterial infection. He introduced
intensely rigorous test conditions that would eliminate all but the most effective compounds.
This involved screening compounds on mice inoculated with a highly virulent strain of
haemolytic streptococcus, i.e. *Streptococcus pyogenes*. The commonest diseases caused by this
organism were tonsillitis and scarlet fever, from which most patients recovered uneventfully.
However, the streptococci sometimes invaded the middle ear to cause otitis media, resulting in
permanent deafness. Occasionally, fatal meningitis also resulted. Further complications of
infection with the haemolytic streptococcus included rheumatic fever and acute nephritis, both
of which could also be fatal. During the worldwide influenza epidemic of 1918–1919,
pneumonia caused by this organism was a common cause of death. It was also responsible for
many fatalities after wounding, both mild and severe, during the First World War. Burning
and scalding were particularly likely to be followed by haemolytic streptococcal infection.
Whatever the original cause of infection, the appearance of haemolytic streptococci in the
blood of a patient, septicaemia, was an ominous sign.

The particular strain of haemolytic streptococci selected by Domagk was isolated from a
patient who had died from septicaemia, and its virulence had been increased by repeatedly
subculturing it in mice. This ensured that the test system was reliable and 100% of the mice
consistently died within four days of inoculation. Only an exceptional drug could influence
such an otherwise inevitable outcome.

Domagk began his investigations by testing three classes of substances that had been
reported as having antibacterial properties, namely gold compounds, acridines and azo dyes.
The first to exhibit activity in the mice were organic gold compounds. Domagk confirmed
their activity and showed that they could even cure larger animals that were similarly infected.
Unfortunately, kidney damage prevented the administration of the dosage required to cure
streptococcal infections in patients. After this setback, Domagk turned his attention to dyes.
The low toxicity of phenazopyridine encouraged Fritz Mietzsch and Josef Klarer to synthesise
analogues of it, so they attached to chrysoidine a side-chain that had previously conferred
antistreptococcal activity on acridines. Domagk found that this greatly enhanced the activity
against cultures of streptococci, but negative results were still obtained in infected mice.
Mietzsch and Klarer next took up an old idea originally tried in 1909 by Heinrich Hörlein, now Director of the Medical Division of I.G. Farbenindustrie. This involved introducing a sulfonamide function into azo dyes in order to enhance their ability to bind to wool. When Domagk tested the sulfonamide derivatives of the most promising of the dyes already examined, they turned out to be almost ineffective against cultures of streptococci. Undaunted by this, he then tested them on infected mice. For the first time in four years since the screening programme had begun, a genuine protective effect was apparent when high doses of one of the dyes were administered. A patent was applied for on 7 November 1931. During the next year a large range of sulfonamide dyes were synthesised and tested. Many of them not only cured the mice in an unprecedented manner, but were also non-toxic. Finally, on Christmas Day 1932, a patent application for another batch of sulfonamide dyes was submitted. Among these was a red dye that was to make medical history.

Early the following year, the medical division of I.G. Farbenindustrie was asked whether there might be a drug that could help a ten month old boy dying from staphylococcal septicæmia. After being informed that the only drug available was intended for use in streptococcal infections, Dr Foerster, the physician treating the infant, was supplied with tablets of Streptozon. Treatment began at once, the boy receiving half a tablet twice daily by mouth. To everyone’s astonishment, he did not die. After four days his temperature gradually lowered to normal and his general condition improved markedly. Treatment was eventually stopped after three weeks and he was discharged from hospital. The case was reported to a meeting of the Düsseldorf Dermatological Society on 17 May 1933 by Foerster.

Several other Rhineland physicians received supplies of Streptozon, and three brief reports citing case histories appeared in German medical journals during 1934. No experimental details or chemical information appeared in print until Domagk’s first publication on the subject appeared in the Deutsche Medizinische Wochenschrift of 15 February 1935. In this he described how small, non-toxic doses of a brick-red sulfonamide dye called Prontosil Rubrum prevented every single mouse that received it by stomach tube from succumbing to an otherwise lethal inoculation of haemolytic streptococci. Thirteen out of fourteen untreated mice died within three to four days. Domagk also explained that Prontosil Rubrum had been able to cure chronic streptococcal infections in rabbits and also alleviated those caused by staphylococci. Prontosil Rubrum was a new name for Streptozon, which was chosen in view of the broader spectrum of antibacterial activity that had been observed. Later, the drug was given the approved name of sulfamidochrysoidine, although this was infrequently used.

Accompanying Domagk’s paper were three others with clinical reports of two years of investigations into the remarkable antistreptococcal action of Prontosil Rubrum. Although the bacteriological work supporting these and the earlier clinical studies left much to be desired, the papers did testify to both the efficacy and safety of the dye, which made it the first truly effective chemotherapeutic agent for any generalised bacterial infection. Prontosil Rubrum was put on the market shortly after the publication of these papers. Surprisingly, it did not create the sensation that might have been expected. There was a conviction in medical circles that chemotherapeutic agents could have little effect against generalised infections. Only clear-cut clinical results could be expected to allay any doubts. That such results quickly became available was principally due to the efforts of Leonard Colebrook at Queen Charlotte’s Maternity Hospital in London. After listening to a lecture on Prontosil Rubrum delivered by Hörlein to the Royal Society of Medicine in London, Colebrook tried to obtain samples of it. With material eventually supplied from France, he confirmed Domagk’s results.
in infected mice, although only after selecting a particularly virulent strain of haemolytic streptococci. In January 1936 he began to use the drug in patients. Shortly after, he received supplies of Prontosil Rubrum® and Prontosil Soluble® from Germany for use in a clinical trial on 38 dangerously ill women. The following June, Colebrook and Kenny reported in the *Lancet* that only three of these patients died. This publication had considerable impact, not least among pharmaceutical companies. Colebrook and his colleagues continued their studies by treating a further 26 seriously ill women, none of whom died.

Domagk was awarded the Nobel Prize for Medicine in 1939 for his discovery of the antibacterial properties of Prontosil Rubrum®. After acknowledging notification of the award he was detained by the Gestapo and persuaded to reject it. This was a direct consequence of Hitler’s rage over the award of the 1936 Nobel Peace Prize to the pacifist journalist Carl von Ossietsky. It was not until two years after the war ended that Domagk was able to travel to Stockholm to receive his medal, by which time the prize money had reverted to the Nobel Foundation. For Domagk, however, the greatest reward must surely have been when, in February 1935, the life of his own daughter, Hildegarde, was saved by Prontosil Rubrum® after she developed a severe septicaemia caused by pricking her finger with a needle.

Apart from the introduction in July 1935 of the now obsolete azosulfamide, an injectable sulfonamide known as Prontosil Soluble®, no other major development in this field came from the I.G. Farbenindustrie laboratories. The initiative passed to French, British, American and Swiss workers – despite the fact that over 1000 sulfonamides had been synthesised at Elberfeld during the five years following the first recognition of antibacterial activity among such compounds.

**Sulfanilamide**

An important report was issued from Fournneau’s laboratory in 1935 by Jacques and Therese Trefouel, Federico Nitti and Daniele Bovet, who suggested that the azo linkage of sulfamidochrysoidine was cleaved in the patient’s body to form 4-aminobenzene sulfonamide, a colourless compound. Fournreau promptly synthesised it as 1162 F, using a method described in the literature in 1908 by Paul Gelmo of Vienna, who had prepared it for his doctoral thesis. Tests quickly revealed that it retained the activity of Prontosil Rubrum®; it was therefore called, for a while, Prontosil Album. The approved name given to this non-patentable compound was sulfanilamide.

Sulfanilamide was then isolated from the urine of patients by Colebrook’s assistant, A.T. Fuller. Hörllein subsequently admitted that his company had already discovered that this was the active form of Prontosil Rubrum®, but had considered it possible that the unmetabolised Prontosil Rubrum® stimulated the immune system to fight off infection. This is significant because there has been speculation that the two-year delay (unprecedented in those days!) by I.G. Farbenindustrie in bringing Prontosil Rubrum® on to the market could have been caused
by its efforts to find some way of protecting their discovery from exploitation by rival manufacturers once it was known that the active species was the non-patentable sulfanilamide. The company’s reply to this was that careful validation of their clinical results was necessary since they were both unprecedented and unlikely to be believed – as was indeed the case. Hörlein claimed they were also trying to establish whether the intact drug was an immunostimulant. This could explain the concentration of effort by I.G. Farbenindustrie on the development of analogues of Prontosil Rubrum\(^1\) rather than analogues of sulfanilamide and tends to support Hörlein’s contention.

In 1937 an elixir of sulfanilamide was put on sale in the United States. It contained 10% of diethylene glycol as a solvent to render the sulfonamide soluble. During the two months that this preparation was on sale, 107 people died from severe damage to the liver and kidneys caused by the solvent.\(^3\)\(^9\) The chemist who devised the formulation committed suicide. As a consequence of this episode, the US Congress enacted a Food and Drug Act in an attempt to prevent such a state of affairs ever arising again. That the United States was spared the thalidomide tragedy 20 years later was one direct consequence of this legislation.

The revelation that sulfanilamide was a systemically active antibacterial agent caught the attention of several companies. The first of its analogues was marketed in 1938 by Schering–Kahlbaum of Berlin, namely sulfacetamide.\(^4\)\(^0\) It was a stronger acid (\(pK_a 5.4\)) than most other sulfonamide drugs because of resonance stabilisation of its anion. This meant that a greater proportion was ionised in the glomerular filtrate, causing it to be rapidly excreted by the kidneys rather than reabsorbed through the renal tubules. While this was undesirable for systemic therapy, it did guarantee the high urinary levels that for many years ensured its use in the treatment of urinary tract infections.

Another consequence of the enhanced acid strength of sulfacetamide was that solutions of its sodium salt were not as alkaline as those of other sulfonamides, rendering them ideal for application to the eye, a purpose for which it was widely employed for many years despite lack of any evidence of efficacy. This use has been abandoned.

### Heterocyclic Sulfonamides

The most important early development following the introduction of sulfanilamide was the synthesis of sulfapyridine.\(^4\)\(^1\) This was carried out in 1937 at the suggestion of Arthur Ewins, the Director of Research at the May and Baker laboratories in Dagenham, London, who wanted to study the action of sulfonamides substituted with a heterocyclic ring on the sulfonamide nitrogen. Sulfapyridine proved to be not only more potent than sulfanilamide but it also had a wider spectrum of antibacterial activity, being effective against pneumococci, meningococci, gonococci and other organisms. As a result, it was supplied to Lionel Whitby at the Middlesex Hospital in London, where he first established that it had unprecedented efficacy against mice inoculated with pneumococci, despite being less toxic than sulfanilamide. He then organised a clinical trial in which sulfapyridine fully lived up to its early promise by reducing the mortality rate among patients with lobar pneumonia from 1 in 4 to only 1 in 25.\(^4\)\(^2\)

At a stroke, this dreaded disease was no longer to be one of the commonest causes of death among otherwise healthy adults. One whose life was saved was Winston Churchill, when he contracted pneumonia during his wartime visit to North Africa in December 1943. This event had as great an impact on the popular imagination as had the anaesthetising of Queen Victoria with chloroform some 90 years earlier. The proprietary name of M & B 693\(^\circ\) at once became
world famous, though few realised that the life of the British Prime Minister had been saved through research initiated in Germany. However, increasing bacterial resistance caused by extensive overprescribing of sulfapyridine and other sulfonamides eventually led to their replacement by antibiotics, most of which had fewer side effects. Typical side effects of the sulfonamides included rashes, renal damage owing to their insolubility and blood dyscrasias. The principal use of sulfonamides at present is in the treatment of urinary tract infections.

Following the introduction of sulfapyridine, varieties of heterocyclic sulfonamides were developed in different laboratories during the early 1940s, often involving patent conflicts between rival companies rushing to market their products. Few of them offered any advantage over sulfapyridine other than perhaps a reduced tendency to deposit crystals in the kidneys. This nasty side effect was due not only to the inherent insolubility of the sulfonamides but also to that of their $N^4$-acetyl metabolites (i.e. where the acetyl substituent is attached to the aromatic nitrogen atom). This insolubility was attributable to intermolecular H-bonding. The $N^4$-acetyl metabolites, which were usually the major ones, were devoid of antibacterial activity.

The risk of crystals depositing in the kidneys was diminished by encouraging patients to drink plenty of fluids or by raising the urinary pH to increase the proportion of water-soluble ionised species in the glomerular filtrate. This was achieved by giving potassium citrate or sodium bicarbonate by mouth until the urinary pH reached about 7.

The problem of crystals depositing in the kidneys was finally overcome by the introduction of sulfonamides that were more highly ionised in the urine because they were stronger acids. There was a limit to the degree of acidity that could be considered since compounds with a $pK_a$ value as low as 5–6 were very rapidly excreted by the kidneys, as in the case of sulfacetamide. The ideal sulfonamides for treating systemic infections required a $pK_a$ value in the range of 6.5–7.5 in order to balance the risk of kidney damage against rapid excretion. Two drugs, in particular, came within this range and both remain in use for the parenteral treatment of meningococcal meningitis, viz. sulfadiazine ($pK_a$ 6.52) and sulfadimidine ($pK_a$ 7.4).

Sulfadiazine, prepared in 1940 by Richard Roblin and his colleagues at the Stamford Research Laboratories of the American Cyanamid Company, was not only more potent than sulfapyridine but also less toxic. Furthermore, it had a wider spectrum of activity than any previous sulfonamide, hence it was used extensively during the Second World War. Sulfadimidine is also known as sulfamezathine and was first synthesized at Temple University in Philadelphia by William Caldwell and two of his Masters students. It had even greater solubility in urine than sulfadiazine, though it was less potent.

**DRUGS DERIVED FROM THE ANTIBACTERIAL SULFONAMIDES**

The relative ease with which sulfonamides could be synthesised from commercially available 4-aminobenzensulfonyl chloride encouraged many companies to venture into this area, with
the result that several classes of novel chemotherapeutic agents were discovered. However, this was not the sole stimulus to drug research that evolved from the introduction of the sulfonamides. Their side effects caused many problems in the clinic, but were successfully exploited to provide oral antidiabetic drugs and valuable diuretics that have benefited many patients with cardiovascular disease.

Antileprotic drugs

Arthur Buttle and his colleagues at the Wellcome Research Laboratories in London investigated 4:4'-diaminodiphenylsulfone as a potential analogue of sulfanilamide. It had been synthesised in 1908 at the University of Freiburg and was later to become known as dapsone. Although it was found to be 30 times more potent than sulfanilamide when tested on mice infected with streptococci, it was 15 times as toxic.

Several derivatives of dapsone were made in different laboratories in the hope of finding safer sulfones. Among them was a water-soluble analogue, glucosulfone, synthesised in the laboratories of Parke, Davis and Company. It initially appeared to be safer than dapsone, so samples were sent to the Mayo Clinic where it was found to be active against \textit{Mycobacterium tuberculosis} in guinea pigs. Guy Faget of the US National Leprosarium in Carville, Louisiana, was given details about this work after he contacted Parke, Davis for information about their new drug. His interest in it arose from his view that any drug effective against tuberculosis could also have value in leprosy, as both diseases were caused by a mycobacterium. Parke, Davis also put Faget in touch with Edmund Cowdry at Washington University School of Medicine in St Louis, Missouri, who was evaluating glucosulfone in rats infected with \textit{M. leprae}, the causative organism of leprosy. Cowdry informed Faget that glucosulfone not only reduced the size of the lesions in rats but also brought about an improvement in their general physical condition. The results were published in 1941. By this time Cowdry’s work had convinced Faget that studies on humans should begin. With the support of Parke, Davis, he tested glucosulfone on six volunteers at the National Leprosarium. Early signs that the drug was effective led to the setting up of a controlled clinical trial of glucosulfone and sulfoxone sodium, a sulfone synthesised by Hugo Bauer of Abbott Laboratories. This concluded that both drugs were effective in the treatment of leprosy, although severe side effects could occur. Further trials around the world confirmed that this was the long-awaited breakthrough in treating leprosy.

After the clinical trials on glucosulfone sodium had been reported, investigations into the value of dapsone revealed that it was as effective as any of the sulfones derived from it for
treating leprosy. Despite problems with resistance, it remains in use today as the standard antileprotic sulfone, while its analogues have been largely abandoned.

Diuretics

At the University of Cambridge in 1940, Thaddeus Mann and David Keilin carried out an experiment to determine whether the fall in carbon dioxide binding power of the blood caused by some of the recently discovered antibacterial sulfonamides could be accounted for by inhibition of carbonic anhydrase. This enzyme, which they had isolated in a pure state a year before, was known to play an important role in the output of carbon dioxide by the lungs. The experiment confirmed their suspicions. However, only those sulfonamides in which both hydrogen atoms on the sulfonamide function were unsubstituted were enzyme inhibitors. These included sulfanilamide and seven sulfonamides devoid of antibacterial activity. Horace Davenport at the Harvard Medical School then discovered large amounts of carbonic anhydrase in the kidneys, leading Rudolf Höber to suggest that the alkaline diuresis in patients who had been given massive doses of sulfanilamide might be accounted for by increased excretion of sodium bicarbonate caused by carbonic anhydrase inhibition. It had been shown, shortly before this, that resorption of water from the tubules of the kidney depended principally on the absorption of sodium ions from the lumen. Subsequently, it was established that carbonic anhydrase promoted the exchange of sodium for hydrogen ions in the distal portion of the renal tubules. When the enzyme was inhibited, sodium ions were excreted in the urine because the process responsible for their reabsorption was blocked.

Davenport next sought a more potent inhibitor of carbonic anhydrase from Richard Roblin at the Lederle Division of the American Cyanamid Company. Thiophen-2-sulfonamide was provided in the belief that it would be more acidic than conventional sulfonamides and that this would enhance its ability to compete with carbon dioxide for the active site on the enzyme. When tested, it proved to be about 40 times more potent an inhibitor than sulfanilamide, as a consequence of which Davenport recommended it as more reliable for investigating the role of carbonic anhydrase in the tissues.

In 1949, Boston physician William Schwartz administered large doses of sulfanilamide by mouth to obtain a diuretic effect in three patients with congestive heart failure, but he had to abandon this approach because of toxic side effects. However, his report rekindled Roblin’s interest in carbonic anhydrase inhibitors. He and James Clapp set about synthesising some 20 heterocyclic sulfonamides and, within a year, acetazolamide was found to be around 330 times more potent than sulfanilamide as an inhibitor of the enzyme. It was introduced clinically as an orally active diuretic in 1952, but inhibition of carbonic anhydrase throughout the body led to a variety of complications. The only acceptable way to use acetazolamide as a diuretic was on an intermittent schedule. Happily, the inhibition of carbonic anhydrase in other parts of the body was turned to advantage in the treatment of glaucoma. By acting on the aqueous humour of the eye in much the same way as in the kidneys, namely to reduce bicarbonate levels and the water secreted with it, the build-up of pressure from excess fluid was overcome. Acetazolamide remains in use for this purpose.

Leading a new project for Merck, Sharp and Dohme, Karl Beyer considered that the problem with sulfanilamide as a diuretic for clinical use was essentially that it inhibited carbonic anhydrase at the distal end of the renal tubules, rather than solely at the proximal end. This, he believed, accounted for the increased excretion of bicarbonate. He sought a
carbonic anhydrase inhibitor that acted in the proximal portion, as indicated by increased excretion of chloride in the form of sodium chloride. Such a drug might, he hoped, have the added bonus of being a useful antihypertensive agent, for clinicians were beginning to believe that low salt diets were an effective means of controlling high blood pressure. To identify a saluretic drug of this type, Beyer carried out salt assays on urine collected from specially trained dogs. These assays were done almost instantaneously by means of flame photometry.

The first carbonic anhydrase inhibitor that Beyer found to increase chloride excretion was 4-sulfonamidobenzoic acid, which received the approved name of carzenide.\(^{60}\) It was a cheap by-product from the synthesis of saccharin, which had been shown to be a weak carbonic anhydrase inhibitor by Hans Krebs.\(^{61}\) Carzenide still increased bicarbonate excretion, indicating a lack of specificity of action within the kidneys. In humans, it was poorly absorbed from the gut and had weak diuretic activity. Nevertheless, James Sprague and Frederick Novello were encouraged by it to synthesise more aromatic sulfonamides for Beyer and his associates to test. They then found that when a second sulfonamido group was introduced, chloride ion excretion was markedly increased.\(^{62}\) Further enhancement of activity followed on the introduction of a chlorine atom into the benzene ring. The outcome was the discovery that clofenamide was a potent carbonic anhydrase inhibitor. As it was a known compound,\(^{63}\) clofenamide could not be patented, but that did not apply to the dichlorphenamide.\(^{64}\) Unlike acetazolamide, it produced an increase in chloride secretion in man.\(^{65}\)

Merck researchers found that when an amino group was attached to the benzene ring of dichlorphenamide, there was a reduction in carbonic anhydrase inhibitory potency. Surprisingly, there was no corresponding reduction in chloride ion excretion. This proved to be a major breakthrough towards the goal of obtaining a saluretic agent. Novello proceeded to synthesise analogues with substituents on the amino group. In the course of this, he tried to make the N-formyl analogue with formic acid. This resulted in an unplanned ring closure to form a benzothiadiazide. As a matter of routine, this novel compound was entered in the screening programme. One can well imagine the surprise and delight when it was found to be a potent diuretic which did not increase bicarbonate excretion. Clinical tests confirmed that it was a safe, orally active diuretic with marked saluretic activity. The first reports appeared in 1957, and it was given the name ‘chlorothiazide’.\(^{66}\) It had a short duration of action of 6–12 hours. Chlorothiazide remains in use because of its low price.

Chlorothiazide was the first of many thiazide diuretics. Literally overnight, it rendered mercurial diuretics obsolete for the treatment of cardiac oedema associated with congestive heart failure. However, that was not all. Beyer was correct in his long-held belief that a safe diuretic that could increase sodium chloride excretion would be of value in the treatment of hypertension. Thiazide diuretics and related compounds are still used for this purpose.
Ciba scientists led by George De Stevens replaced the formic acid used to produce chlorothiazide with formaldehyde and thereby obtained hydrochlorothiazide, which was ten times as potent as chlorothiazide.\textsuperscript{67} At least four American companies then synthesised hydroflumethiazide, which had similar properties to hydrochlorothiazide.\textsuperscript{68-71} Bendrofluazide was synthesised at the same time as hydroflumethiazide.\textsuperscript{68} Its action lasted 18–24 hours. As one of the cheapest diuretics on the market, it remains widely used in patients with either mild heart failure or hypertension. Many other thiazides have been developed.

The realisation that the second acidic group in dichlorphenamide may be replaced with a carboxyl group, so long as an appropriate substituent is present on the amino group, led Hoechst to introduce frusemide (also known as ‘furosemide’) in 1962.\textsuperscript{72} It had a quicker onset of activity, within one hour of oral administration, which was more intense and of shorter duration than that of other diuretics.\textsuperscript{73}

Frusemide had a different site of action within the kidney tubule and became known as a loop diuretic because it acted in the region known as the loop of Henle. Loop diuretics were valuable in patients with pulmonary oedema arising from left ventricular failure. Despite thiazides being indicated for most patients requiring a diuretic, frusemide is widely prescribed.

Bumetanide is a more potent loop diuretic introduced by Leo researchers ten years after frusemide.\textsuperscript{74} Hoechst introduced its analogue known as piretanide when their patent on frusemide expired.\textsuperscript{75}

**Antidiabetic Sulfonylureas**

In March 1942, Marcel Janbon arranged a clinical trial at Montpellier University of 2254 RP, an experimental sulfonamide, in patients with typhoid.\textsuperscript{76} Some of them became very ill, and a few died. The survivors made a startling recovery after receiving intravenous glucose, which led to suspicion that the drug had been producing severe hypoglycaemia. When this was confirmed, Janbon asked August Loubatieres to conduct a full investigation into the effects of the drug on animals for his doctoral studies. Although Loubatieres concluded that 2254 RP could be of value in diabetes, this suggestion was ignored.\textsuperscript{77}
When a sulfonylurea developed by the C.H. Boehringer Company as a long-acting sulfonamide was put on clinical trial at the Auguste Viktoria Hospital in Berlin in 1954, it produced severe toxic effects. On testing the drug on himself, Joachim Fuchs found that it produced the symptoms of severe hypoglycaemia. The chief of his clinic, Hans Franke, conducted further investigations that led to the introduction of the drug as an oral hypoglycaemic agent with the approved name of carbutamide.\(^7\) When the Eli Lilly Company arranged extensive clinical trials of it in the United States, the incidence of side effects was unacceptable, even though the drug was already being used in Europe. Upjohn meantime arranged a trial of Hoechst’s closely related tolbutamide,\(^7\) involving 20,000 patients and 3,000 doctors. This time the drug received approval from the Food and Drug Administration for use in type 2 (non-insulin-dependent) diabetes. Unlike carbutamide, it did not possess antibacterial properties, so there was no likelihood of inducing resistant bacteria. It had to be taken three times a day because the methyl group was rapidly metabolised to a carboxylic acid.

Soon after the introduction of tolbutamide, Pfizer marketed chlorpropamide.\(^8\) As it did not feature the metabolically sensitive methyl group of tolbutamide, it was about twice as potent as tolbutamide and could be taken once daily. While initially welcomed, this feature has been found to be disadvantageous in elderly patients in whom the drug can accumulate and thereby produce hypoglycaemia. Other longer-acting sulfonylureas have been marketed, including highly potent agents such as glibenclamide (glyburide)\(^8\) and glipizide.\(^9\)

### Proguanil

On the outbreak of the Second World War, a high-priority rating was given to a scheme established by the British Medical Research Council to develop new antimalarial drugs. This
resulted in a harmonious collaboration between 20 leading academics and a similar number of industrial chemists, involving the synthesis of around 1700 novel compounds, of which one-third exhibited antimalarial activity against experimental infections. One of the companies involved was ICI, where Francis Rose followed up evidence that sulfadimidine had weak activity against malaria. Attributing this to the presence of the pyrimidine ring, he and his colleagues synthesised a range of pyrimidines incorporating features present in mepacrine.

Compound 2666, which contained a basic side chain and a chlorophenyl moiety, was active in chickens infected with *Plasmodium gallinaceum*. The most effective of its analogues were biguanides in which the pyrimidine ring was split open. The first of the biguanides to be screened had been devoid of antimalarial activity, but it was realised that this could have been due to the presence of too many basic nitrogenous side chains. Replacement of one of these by an isopropyl group led to the reappearance of strong antimalarial activity. Around 200 biguanides were synthesised and tested before proguanil emerged as being superior to mepacrine.83

Clinical trials at the Liverpool School of Tropical Medicine confirmed that proguanil was a first-line drug for the treatment of the erythrocytic phase of malaria, although it is now used mainly for the prophylaxis of malaria in those parts of the world where the parasites have not yet developed resistance.

Quinolones

In 1946, Alexander Surrey and H.F. Hammer of the Sterling–Winthrop Research Institute in Rensselaer, New York, devised a novel synthesis of chloroquine which produced a by-product, viz. 7-chloro-1,4-dihydro-1-ethyl-4-oxoquinoline-3-carboxylic acid. Some years later, this by-product was included in a screening programme and was found to be effective against fowl coccidiosis. When analogues were prepared by George Lescher and his colleagues, nalidixic acid emerged as a potent antibacterial agent effective against Gram-negative rods, with no cross-resistance to other antibiotics then in routine use.84 Being a polar compound it was rapidly excreted by the kidneys, hence adequate tissue levels of drug could not be achieved. This, however, was ingeniously exploited when nalidixic acid was introduced as a urinary antiseptic in 1962. It was initially believed to bind to the A subunit of bacterial DNA gyrase, thereby interfering with the supercoiling of chromosomes required for them to be packed sufficiently tightly for cell replication to proceed. However, it is now known that nalidixic acid and its quinolone analogues bind to topoisomerase IV.85

Analogues of nalidixic acid were investigated in the hope of widening the spectrum of action. Early ones have been described as ‘second-generation quinolones’. While the 3-carboxyl and 4-oxo groups were essential for activity, it was possible to alter the heterocyclic
ring. One of the first compounds to exhibit superior activity was Warner–Lambert’s oxolinic acid, which was found to be more potent than nalidixic acid. It was rapidly excreted in the urine, once again limiting its use to the treatment of urinary tract infections. Lescher synthesised acrosoxacin, which was about 10 times as potent as nalidixic acid and had a similar spectrum of activity. Its principal value was in the treatment of gonorrhoea in patients who were either allergic to penicillins or infected by strains resistant to other antibiotics.

Several quinolones were developed in Japan. Norfloxacin was prepared in 1978 by the Kyorin Pharmaceutical Company in Tokyo. The combination of the piperazine ring at the 7-position of the quinolone ring and the fluorine atom at the 6-position had the effect of radically altering the spectrum of activity, which was widened to include in a quinolone for the first time a low level of activity against Gram-positive organisms. Unfortunately, norfloxacin was poorly absorbed from the gut, with only about one-third of a dose entering the circulation. It then underwent both hepatic oxidation and renal elimination, hence it could not be considered for anything other than treatment of gastrointestinal or urinary tract infections. However, it served as the lead for further development of the fluoroquinolones.

Researchers at the Dainippon Pharmaceutical Company in Osaka introduced an extra hetero-nitrogen atom to produce enoxacin, which by good fortune provided better oral bioavailability than that found with norfloxacin. Ofloxacin is another quinolone developed in Japan, having been synthesised by the Daiichi Seiyaku Company in Tokyo. It had a spectrum of activity and absorption profile comparable to that of enoxacin, but was resistant to metabolic oxidation in the liver. It is mainly used in urinary tract infections, gonorrhoea and respiratory infections. The S(–) stereoisomer is also available as levofloxacin. The still more potent quinolone ciprofloxacin was introduced by Bayer AG in 1987. Several more fluoroquinolones have been developed since then, including grepafloxacin, alatrofloxacin, sparfloxyacin and trovafloxacin.

Antituberculous Drugs

In 1938, Arnold Rich and Richard Follis at Johns Hopkins Hospital reported that sulfanilamide had weak activity in animals infected with *Mycobacterium tuberculosis*. The following year, Gerhardt Domagk found that sulfathiazole and the related sulfadithiazole were much more effective. Domagk also screened the thiosemicarbazides used in the synthesis of the sulfadithiazoles. To his surprise, they were more active than the sulfonamides, benzaldehyde thiosemicarbazone being particularly so. A series of thiosemicarbazones were
then prepared for Domagk to test, from which thiacetazone emerged as a potential therapeutic agent. Clinical trials took place in Germany at the end of the war during an epidemic of tuberculosis, but were inadequately organised and led to unrealistic claims being made. American physicians subsequently discovered that thiacetazone was too toxic to the liver for routine therapeutic application.

When researchers at Lederle Laboratories reported in 1948 that nicotinamide had mild tuberculostatic activity, it caught the attention of both Domagk in Germany as well as Robert Schnitzer of Hoffmann–La Roche laboratories in Nutley, New Jersey, where Hyman Fox had made several pyridine derivatives with antituberculous activity. Both men immediately recognized the possibility of combining a nicotinamide residue with a thiosemicarbazone to form isonicotinaldehyde thiosemicarbazone. Each of them subsequently discovered that the chemical intermediate used in the synthesis of the thiosemicarbazone was itself a highly potent antituberculous drug. It was tested in New York hospitals and quickly became established as the most valuable antituberculous drug ever discovered. It received the approved name of isoniazid and remains in use as an essential component of the combinations of drugs used to treat this deadly disease, which has reappeared with renewed virulence in recent years.

Shortly after the announcement of the discovery of the activity of isoniazid, both Kushner of Lederle Laboratories and Solotorovsky of Merck and Company simultaneously reported the antituberculosis properties of the nicotinamide analogue known as pyrazinamide. This remains an important drug because of its efficiency in meningeal penetration in cases of meningeal tuberculosis. The Theraplix company in Paris subsequently introduced ethionamide, but it is now rarely used.

Antidepressant Drugs

When a clinical trial on an analogue of isoniazid developed by Hoffmann–LaRoche was conducted at Sea View Hospital on Staten Island, there was concern about its side effects, particularly central nervous system stimulation. This particular property of the new drug, iproniazid, was then pursued elsewhere. At a meeting of the American Psychiatric Association in Syracuse, New York, in April 1957 there were several reports of the value of iproniazid in depression, including one from a group of psychiatrists led by Nathan Kline of Rockland State Hospital, Orangeburg, New York. He presented the meeting with results that revealed iproniazid to have been the first drug of value in chronically depressed psychotic patients. Kline had begun to study the effects of iproniazid after being shown the results of animal experiments carried out by Charles Scott of the Warner–Lambert Research Laboratories in New Jersey. At that time it was known that reserpine caused brain cells to liberate 5-HT and noradrenaline. It was suspected that the tranquilising effect might be due to the release of the former, so Scott administered iproniazid to inhibit the enzymatic destruction of this substance. That iproniazid could inhibit the enzyme, monoamine oxidase, had been known since 1952.
To Scott’s surprise, pre-medication of animals with iproniazid before administration of reserpine caused stimulation, rather than the expected tranquilisation. When Kline saw the results of the animal studies, he carried out similar studies on humans. He then found that iproniazid on its own could stimulate depressed patients.

Since iproniazid had already been marketed as an antituberculous drug, psychiatrists were able to obtain supplies as soon as they heard of its antidepressant properties. More than 400 000 patients received it for depression during the year after the Syracuse conference, but Hoffmann–LaRoche withdrew iproniazid from the American market after a number of cases of jaundice had been reported. They replaced it with isocarboxazid, which was a more potent monoamine oxidase inhibitor.103 This remains in use for treatment of patients who have not responded to other antidepressants. As it inhibits all types of monoamine oxidases, isocarboxazid may produce life-threatening hypertension after eating foods and wines rich in tyramine, a pressor amine. Warner–Lambert’s phenelzine had similar properties to isocarboxazid.104

When the first attempts to treat Parkinson’s disease with levodopa were made, it was realised that much of the drug was metabolised before it could reach the brain. Alfred Pletscher and his colleagues at the Hoffmann–LaRoche laboratories in Switzerland then investigated the possibility of finding an inhibitor of the enzyme responsible, DOPA decarboxylase.105 They found that benserazide, a compound synthesised as a potential monoamine oxidase inhibitor and which did not enter the brain, was capable of inhibiting extra-cerebral DOPA decarboxylase to bring about a large reduction in the dose of levodopa required by patients. A combined formulation of both drugs was then marketed.

The liver toxicity following prolonged therapy with iproniazid ensured that Hoffmann–LaRoche investigators in Basle would examine its potential successors very thoroughly. This led to the discovery that 1-methyl-2-benzylhydrazine had a pronounced tumour inhibitory effect. Screening of several hundred of its analogues that had been synthesised as potential antidepressants revealed that 40 methylhydrazines were active antitumour agents. Two of them were selected for extended biological and clinical trials. In 1963, these revealed the value of procarbazine.106 It was subsequently used in combination with mustine, vincristine and prednisolone in the ‘MOPP’ regimen (i.e. mustine + vincristine [Oncovin®] + procarbazine + prednisone), which transformed the prospects for survival of patients with advanced Hodgkin’s disease.

**Inhibitors of Gastric Acid Release**

At the Hassle division of Astra, an analogue of ethionamide known as ‘pyridylthioacetamide’ was found to inhibit gastric acid secretion when it was routinely included in a screening test using dogs with chronic gastric fistulas.
Because of the toxicity known to be associated with a thioacetamide function, other sulfur compounds were examined. This led to the discovery of antisecretory activity in H 77/67. Structural variants of this were prepared, culminating in the discovery of a potent antisecretory benzimidazole, compound H 124/26, just over one year later. This underwent metabolism to the more potent sulfoxide, subsequently called timoprazole. When this was submitted to chronic toxicity testing it was found to prevent uptake of iodine by the thyroid, so further analogues had to be prepared and tested for both antisecretory and antithyroid activity. Picoprazole was synthesised in 1977 and found to be safe and effective. More potent analogues were then obtained by increasing the pK_a of the pyridine ring by placing electron-donating groups on to it. One of the promising results of this was H 159/69, but this ester proved too unstable for clinical use. Modification of it led to omeprazole, in 1979. This proved to be a safe, potent inhibitor of gastric acid secretion and soon rivalled cimetidine and ranitidine in the marketplace.

Aminosalicylates for Bowel Disorders

At the Karolinska Institute in Stockholm, Nanna Svartz began experimenting on the treatment of rheumatoid arthritis in 1938. Following the introduction of sulfapyridine, he experimented with it to eradicate a postulated diplostreptococcal infection which he believed was a causative factor in both rheumatoid arthritis and ulcerative colitis. When no improvement occurred in his patients, he tried to combine sulfapyridine with salicylic acid to form a drug that would possess the ability of the latter to target the connective tissue while possessing antistreptococcal activity. Failing to achieve a successful synthesis, he turned to the Pharmacia Company for assistance. The company arranged for Willstedt, their chemical consultant from the University of Stockholm, to prepare four test compounds for Svartz. One of these was sulfasalazine, which was administered to a patient suffering from ulcerative colitis. Within days, the patient became symptom free and the diarrhoea ceased. More patients received the treatment and those with ulcerative colitis responded well, but only some of the arthritic patients improved. Subsequent studies confirmed that sulfasalazine concentrated in connective tissue and in the lumen of
the intestine. Bacterial azoreductase enzymes in the colon then metabolised it to release mesalazine, the active anti-inflammatory agent. Sulfasalazine became the drug of choice for treatment and prophylaxis of ulcerative colitis, but newer analogues have proved to be safer by avoiding side effects from the sulfapyridine moiety, which has no role to play as streptococci are not involved in the aetiology of ulcerative colitis. One of these is mesalazine, the active metabolite of sulfasalazine.

Olsalazine is a dimer of mesalazine that was once used as a dye called ‘Mordant Yellow’. Pharmacia patented it in 1981 for use in ulcerative colitis. It is activated in the colon in the same manner as sulfasalazine.

REFERENCES


64. US Pat. 1958: 2835702 (to Merck & Co.).


Drugs Originating from the Screening of Organic Chemicals

The previous chapter dealt with a variety of drugs that can trace their origins to the screening of synthetic dyes for chemotherapeutic activity. Even more drugs have been discovered through the screening of other synthetic compounds.

Screening has been used both to discover drug prototypes and to find improved analogues of other compounds that exhibit useful activity but require enhancement in some manner. Examples of the latter process can be found throughout this book, but the present chapter is concerned solely with the discovery of drug prototypes through screening and their subsequent development to provide therapeutic agents.

CLASSICAL ANTIHISTAMINES

After the presence of histamine in the body had been established there was considerable interest in its physiological role. Daniel Bovet at the Pasteur Institute realised that antagonists of acetylcholine and adrenaline, e.g. atropine and ergotamine, had made it possible for physiologists to investigate and understand their actions. Since no antagonist of histamine existed, Bovet screened compounds that had previously been synthesised at the Institute and found that certain adrenaline analogues and antagonists diminished the action of histamine on the guinea pig intestine. However, when the most promising of these, piperoxan, was injected into live guinea pigs it failed to protect them against the lethal effects of histamine administered by the intrajugular route.

On examining similar compounds prepared at the Institute, Bovet and Anne-Marie Staub found Ernest Fourneau’s compound 929F to be the most potent histamine antagonist yet tested on the guinea pig intestine. When it was administered to guinea pigs, it consistently protected them against the otherwise lethal bronchoconstrictive action of histamine. Compound 1167F, in which the oxygen atom was replaced by nitrogen, was also effective.

Staub investigated several more compounds and proceeded to define the molecular requirements for antihistaminic activity, with remarkable accuracy. Unfortunately, none of the compounds examined were safe enough for human administration. It was not until 1941...
that phenbenzamine was found suitable for clinical use after Mosnier had synthesised 24 analogues of 1571F at the Rhône-Poulenc laboratories in Paris.\textsuperscript{5}

\[ \text{phenbenzamine} \quad \text{mepyramine} \quad \text{tripelennamine} \]

Two years after the introduction of phenbenzamine, Bovet and his colleagues published their studies on the closely related mepyramine, in which a pyridine ring replaced one of the benzene rings.\textsuperscript{6} This alteration was probably introduced because of the experience Rhône-Poulenc had acquired from their British subsidiary, May and Baker, who had developed sulfapyridine. Researchers at Ciba Pharmaceuticals in Summit, New Jersey, synthesised tripelennamine, which differed only in the absence of the methoxyl group attached to the benzene ring.\textsuperscript{7}

\[ \text{cyclizine} \quad \text{hydroxyzine} \]

The American division of Burroughs Wellcome developed cyclizine, a long-acting antihistamine in which the amino group was derived from piperazine instead of dimethylamine.\textsuperscript{8} In the clinic it proved to be a useful anti-emetic drug, achieving the notable distinction of being selected by the US National Aeronautic and Space Agency for use as a space sickness remedy on the first manned flight to the moon. The related hydroxyzine is an antihistamine that has also been used as a minor tranquilliser.\textsuperscript{9}

During the Second World War, Rhône-Poulenc followed up Ehrlich’s demonstration of antimalarial activity with methylene blue by investigating phenothiazines. This line of research was abandoned after negative results were obtained, but Bernard Halpern and René Ducrot realised that one of the phenothiazines synthesised by Paul Charpentier was an analogue of phenbenzamine in which its two benzene rings were bridged by a sulfur atom. When tested, it proved to be an antihistamine and was given the name ‘fenethazine’.

\[ \text{fenethazine} \quad \text{promethazine} \]

Promethazine, a derivative of fenethazine synthesised in 1946, had an extra methyl group on the dimethylaminoethyl side chain and turned out to be highly potent and a very long-acting antihistamine.\textsuperscript{10} Charpentier had intended to place the methyl group on the adjacent carbon atom. Had he succeeded, the compound would not have been as successful. It was marketed as an antihistamine, but its ability to cause prolonged central depression also led to its use as a non-prescription hypnotic.
Aminoalkyl Ether Antihistamines

Diphenhydramine was one of several compounds designed to be antispasmodics by George Rieveschl, an assistant professor at the University of Cincinnati. It was synthesised in 1943 by Fred Huber, one of his research students. Parke, Davis and Company tested the new compounds on the guinea pig ileum and found diphenhydramine to be a highly potent antispasmodic. Extensive testing revealed not only that it had an exceptionally high safety margin but also that it was a potent antihistamine. Parke, Davis bought the patent rights from Rieveschl, granting him a 5% royalty on all sales for the next 17 years while the patent lasted. Rieveschl joined Parke, Davis and became Director of Research in 1947, in which role he was responsible for the development of the very similar antihistamine known as ‘orphenadrine’. Both compounds had atropine-like anticholinergic effects, which were more marked in orphenadrine and resulted in its use in the treatment of Parkinson’s disease. In an attempt to develop an analogue of orphenadrine with reduced side effects, the Riker Company synthesised nefopam in 1966. At first, this was thought to be a centrally acting muscle relaxant, but was later shown to be an analgesic. It is used in the management of moderate pain.

Antihistamines became immensely popular during the late 1940s, being hailed in some quarters as miracle drugs! Although their principal use was in the control of certain conditions such as hay fever or urticaria, they were initially believed to be of value for a wide range of ailments, including the common cold. Leading pharmaceutical manufacturers competed vigorously to develop new antihistamines, resulting in the introduction of a plethora of new drugs with little to choose between them, none being free of the tendency to cause drowsiness.

G.D. Searle and Company, a family-owned Chicago pharmaceutical distributor, broke new ground by introducing a formulation of diphenhydramine designed to minimise drowsiness by formulating it with a mild stimulant, namely 8-chlorotheophylline. The resulting salt, dimenhydrinate, did not prevent drowsiness, but it became one of the most profitable antihistamines on the market after it was found to have an unexpected therapeutic action. Samples had been sent to the allergy clinic at Johns Hopkins University in Baltimore for evaluation by Leslie Gay and Paul Carliner. They administered dimenhydrinate to a patient suffering from urticaria. She then discovered that when travelling on a streetcar after having swallowed the drug, she was not car sick – for the first time in years! Tests on other patients who suffered from travel sickness confirmed the apparent value of dimenhydrinate. The matter was reported to Searle, who organised an ambitious clinical trial. On 27 November 1947, the General Ballou sailed from New York to Bremerhaven in Germany. The crossing was particularly rough, yet only 4% of the troops on board who received the drug were sick, in contrast to a quarter of the others who received a placebo. Furthermore, all but 17 of 389 sick soldiers recovered within a couple of hours of receiving dimenhydrinate. Unlike earlier remedies such as hyoscine, the only significant side effect was drowsiness. Searle quickly exploited this before their rivals began to discover that other antihistamines were also effective against motion sickness.
Monoaminopropyl Antihistamines

Research workers at the Schering Corporation realised that all the potent antihistamines contained two aromatic rings joined to either a nitrogen or an oxygen atom. They therefore synthesised a new series in which these rings were instead attached to a carbon atom as this had roughly similar atomic dimensions, being an example of isosteric replacement of an atom. This led to the development of the long-acting antihistamines pheniramine, brompheniramine and chlorpheniramine in 1948, the latter two being more potent.\(^{16}\)

\[
\begin{align*}
\text{pheniramine, } R &= \text{H} \\
\text{brompheniramine, } R &= \text{Br} \\
\text{chlorpheniramine, } R &= \text{Cl}
\end{align*}
\]

A similar approach was adopted around the same time, at the Wellcome Research Laboratories in England, although a few years passed before triprolidine was marketed.\(^{17}\)

Merck introduced a cyclic analogue of pheniramine known as cyproheptadine.\(^{18}\) It had similar properties to chlorpheniramine, but was also of some value in the prophylaxis of migraine due to its ability to act as a 5-HT\(_2\) antagonist. It is now reserved for refractory cases of migraine. Pizotifen proved to be better than cyproheptadine in the prophylactic treatment of migraine.\(^{19,20}\)

The Schering–Plough Corporation developed azatadine as a potential non-sedating antihistamine.\(^{21}\) A pre-clinical behavioural test on cats indicated an absence of central activity, but azatadine turned out to be a typical potent sedating antihistamine when administered to human volunteers.

Non-sedating Antihistamines

Richardson–Merrell chemists synthesised terfenadine in 1973 as a potential tranquilliser, but found it to be inactive as it did not enter the central nervous system. Pharmacologist Richard Kinsolving noticed that it had a resemblance to diphenhydramine and it was tested and then found to be an antihistamine that did not cause sedation.\(^{22}\) Clinical trials confirmed that terfenadine was the first non-sedating antihistamine to have been discovered.
In 1992, the US Food and Drug Administration issued a warning that some patients who took terfenadine might develop a life-threatening ventricular arrhythmia called ‘torsades de pointes’. Use of the drug was ruled out in patients with liver disease as it was not being efficiently metabolised. This problem was overcome when the active metabolite, fexofenadine, was introduced.23

Despite the fact that it had taken almost 40 years to discover a non-sedating antihistamine, several more were introduced soon after the launch of terfenadine. Wellcome marketed a derivative of triprolidine known as ‘acrivastine’ which was developed as a non-sedating antihistamine by incorporating an ionisable side chain to reduce central nervous system penetration.24

Frank Villani at Schering–Plough synthesised potential antihistamines designed to antagonise both histamine H1 and H2 receptors. He hoped that these might have useful anti-ulcer properties. He began by making analogues of azatadine in which the basicity of the piperidine ring nitrogen was reduced by the formation of urea, sulfonamide and carbamate derivatives. The resulting compounds failed to exhibit histamine H2 blocking activity. However, when the ethyl carbamate ester was later screened it had no effect on the central nervous system. Further investigation confirmed that it was a non-sedating antihistamine.25 Attempts were then made to find a longer-acting analogue. When a chlorine atom was placed at the 8-position of the ring system to reduce oxidative metabolism, it increased the duration of action in human volunteers from under 8 hours to permit once-daily medication. Unexpectedly, this modification also increased potency by a factor of four.26 This new 8-chloro analogue was also active when given by mouth. It received the approved name of ‘loratadine’, and was marketed as a non-sedating antihistamine.

Antipsychotic Agents

The phenothiazine tranquillisers were developed as a consequence of studies initiated at the Sidi Abdallah Hospital near Bizerte, Tunisia, in April 1949 by Henri Laborit, a French Navy
surgeon who had been one of the first to use antihistamines to pre-medicate patients undergoing surgery. His concern about the traumatic effects of surgical shock led him to consider the possibility that antihistamines might prevent the capillary hyperpermeability caused by histamine release in patients in shock. He therefore incorporated mepyramine and promethazine in the mixture of drugs he was administering. Over an 8 month period it then became apparent to Laborit that the antihistamines had unusual central actions which were contributing to the antishock action.

The mood of his patients had improved and, particularly in the case of promethazine, they were less anxious and required less morphine. An army psychiatrist confirmed this effect of the drug, but matters rested there for the time being.

On being transferred to the Val-de-Grâce Military Hospital in Paris, Laborit took the opportunity to investigate the central effects of antihistamines in more detail. In collaboration with the anaesthetist Pierre Huguenard, he was able to show that they lowered body temperature and so reduced basal metabolism to produce a reduction in the amount of anaesthetic required during operations. This, in turn, lowered the risk of shock, so once more Laborit turned his attention to the effects of pre-medication on shock. He now experimented with a ‘lytic cocktail’ of drugs to cool the bodies of patients wrapped in ice bags even further.

Laborit visited the manufacturer of promethazine, the Specia Laboratories of Rhône–Poulenc at Vitry-sur-Seine, near Paris, and described his work. In the autumn of 1950 they began a search for a drug that would have an action on the central nervous system that met Laborit’s requirements. Simone Courvoisier screened phenothiazines that Paul Charpentier had synthesised as potential antihistamines, investigating those that were previously rejected because of sedating effects. When the fenethazine analogue now known as promazine proved most interesting despite its low level of antihistaminic activity, Charpentier synthesised analogues of it. One of them was chlorpromazine, a chlorinated derivative prepared in December 1950. It was passed to Courvoisier who identified its outstanding activity and low toxicity.

In the spring of 1951, samples of chlorpromazine were given to Laborit. He confirmed that it was indeed the agent he had long sought. After completing appropriate animal tests, he incorporated the new drug into a ‘lytic cocktail’ in combination with promethazine and the fenethazine analogue known as ethazine for use on patients undergoing surgery. Before long, he observed that not only did they fare better both during and after their operations, due to the antishock action, but they also seemed relaxed and unconcerned with what was happening to them during the normally stressful pre-operative period. The significance of this was not lost on Laborit. He persuaded his psychiatric colleagues at the Val-de-Grâce Hospital to test the mixture on psychotic patients. On 19 January 1952 Joseph Hamon, the Director of the Neuropsychiatric Service, assisted by Jean Paraire and Jean Velluz, began to treat a manic patient who was decidedly agitated until he was given his first injection. At once, he became calm and remained so for several hours. It was recognised that the mixture of drugs was palliative rather than curative, but this did not stop the release of the patient from hospital three weeks later.

However, the psychiatrists at the Val-de-Grâce Hospital did not observe the full effects of chlorpromazine as it was only one component of a mixture, with the dose duly modified to take account of the other two central depressants, promethazine and pethidine. Consequently, they soon abandoned the mixture and returned to using electroshock therapy on their patients.

On learning of the effects of the mixture containing chlorpromazine, Pierre Deniker of the Sainte Anne Hospital in Paris requested samples of chlorpromazine from Rhône–Poulenc for a detailed study of its psychopharmacological action when administered without other drugs.
He and his senior colleague Jean Delay conducted a clinical trial on 38 patients, soon confirming its outstanding value as a tranquilliser for manic, agitated and psychotic patients. Chlorpromazine was found to be a relatively sedating antipsychotic drug, but unlike the then popular sedatives (i.e. central depressant drugs such as hypnotics administered in subhypnotic doses) it did not aggravate disorders of wakeful consciousness. Indeed, mental confusion was alleviated. In schizophrenic patients, chlorpromazine produced a diminution in aggressiveness, agitation and delusion. Particularly characteristic of chlorpromazine was its effect on the central control of movement whereby a type of akinesia, or psychomotor indifference, was induced in patients. This led Delay and Deniker in 1955 to introduce the term ‘neuroleptic’ to describe any antipsychotic drug with this effect.

Chlorpromazine was marketed in France by Rhône–Poulenc in the autumn of 1952. The early observations by French psychiatrists and others became generally accepted, with the result that psychiatry was transformed and psychotic patients were released from the restraints of straight-jackets and locked wards. They were not cured, but the phenothiazines controlled their behaviour. Some critics believe that physical restraint had simply been substituted by chemical restraint. That view remains a minority one. As with all other drugs, problems occurred when insufficient care was taken with their administration. A discussion of these here is inappropriate, but considering that millions of patients have received these drugs, their record is impressive.

The remarkable success of chlorpromazine stimulated rival manufacturers to introduce analogues of it. Many of these compounds had a different substituent incorporated in place of the chlorine atom attached at position 2 of the phenothiazine ring. This was motivated not merely by a desire to circumvent the Rhône–Poulenc patents, but also by a belief that potency was influenced by the electron-withdrawing power of the substituent, a view that has not been upheld. Typical variants included acetyl, methoxyl, nitrile, trifluoromethyl, thioalkyl and dialkylsulfonamide groups. The differences between the scores of phenothiazine tranquillisers that have been introduced into the clinic are less significant than the variability in patient response to any single drug.

The Rhône–Poulenc researchers found that more potent analogues could be obtained by replacing the dimethylamine function on the side chain of chlorpromazine with a piperazine group. This increased side effects involving dopaminergic extrapyramidal pathways in the nervous system, leading to a Parkinson’s disease-like tremor in some patients. This may have been a direct consequence of the reduction of anticholinergic activity arising from the use of smaller doses than for chlorpromazine. Anticholinergic drugs are actually used to treat phenothiazine-induced extrapyramidal tremor. There is, however, a benefit from the diminished anticholinergic response in patients treated with the piperazine compounds, namely that they are less sedating. For this reason they are preferred to other phenothiazines for the prevention and treatment of nausea. The first of the piperazine compounds to be marketed was prochlorperazine.
Fluphenazine had a very similar activity to prochlorperazine, but as it had an alcohol function in the side chain, it was also formulated as either the enanthate (i.e. heptanoate) or decanoate ester in an oily depot injection.\textsuperscript{39} This was injected every 14–28 days for the long-term control of psychotic behaviour. In contrast to fluphenazine, thioridazine had a low potency. However, its anticholinergic activity helped to counter extrapyramidal tremor and was an important advantage for elderly patients. This was to some extent offset by an increased risk of hypotension.

In 1958, Petersen and his colleagues, working with the Danish firm H. Lundbeck, published their first report on the thioxanthenes,\textsuperscript{40,41} a new series of tranquillisers in which the nitrogen of the phenothiazine ring had been isosterically replaced by a carbon atom. As these tricyclic compounds had strong chemical similarities to the phenothiazine tranquillisers, it is hardly surprising that their therapeutic activity proved to be similar. The first member of the series to be introduced underwent a clinical trial with 70 patients in 1958 and was marketed the following year with the approved name of chlorprothixene.\textsuperscript{42} Lundbeck introduced clopenthixol three years later after clinical evaluation had shown it to be a better antipsychotic agent than chlorprothixene. It was a mixture of \textit{cis} and \textit{trans} isomers. The active \textit{cis} isomer was introduced into the clinic as zuclopenthixol.\textsuperscript{43} The acetate ester was also developed for depot medication.

Reports of persistent abnormal facial movements among patients who had taken chlorpromazine began to be published within five years of its introduction.\textsuperscript{44} The condition was termed ‘tardive dyskinesia’. Lawsuits were brought against companies that marketed the drug. Once it was realised that related drugs could also cause tardive dyskinesia, companies were discouraged from developing new tranquillisers.

**Anxiolytic Drugs**

Early in 1954 at the laboratories of Hoffmann–LaRoche in Nutley, New Jersey, Leo Sternbach decided to reinvestigate some tricyclic compounds he had synthesised about 20 years earlier at the University of Cracow as part of his post-doctoral studies on dyestuffs. He had in mind the tricyclic nature of chlorpromazine, which had just been discovered, and he believed that the introduction of a basic side chain into his own compounds might create derivatives with a degree of overall similarity to it. He prepared around 40 new compounds by reacting his key intermediate, an alkyl halide, with a variety of secondary amines selected to confer structural analogy with the tricyclics then being patented. When these compounds were submitted to Lowell Randall for screening for muscle relaxant, sedative and anticonvulsant properties, they were all found to be inactive. Renewed chemical studies then revealed that the tricyclic system of the key synthetic intermediate was not that of a benzheptoxdiazine, as had been believed, but was instead a quinazoline-3-oxide. This seemed to account for the lack of biological activity in the derivatives synthesised from this intermediate. The last compound Sternbach had prepared remained untested until a year and a half later, when a colleague who was tidying up the laboratory suggested it should be sent for screening. Sternbach agreed, and a few days later Randall informed him that his compound appeared to approach the activity of chlorpromazine as a tranquilliser. Furthermore, it had a low level of acute toxicity and was free from significant side effects. This report engendered considerable excitement and raised
the obvious question of why only this single compound was active. The answer was soon found when Sternbach reinvestigated its chemistry. It became clear that by using the primary amine methylamine in the last stage of the synthesis, the reaction had followed a different pathway (ring enlargement) from that undergone when secondary amines had been employed. The product formed was a benzodiazepine.\textsuperscript{45,46} Sternbach filed a US patent application for this new tranquilliser, chlordiazepoxide, in May 1958. The initial clinical studies were conducted on 16 000 patients before it was granted approval by the US Food and Drug Administration in 1960. Thousands of benzodiazepines have been synthesised since then, of which several are still used throughout the world as anti-anxiety agents and hypnotics. While they can be of value in patients whose anxiety interferes with their work, leisure and personal relationships, the benzodiazepines have been widely misused in the treatment of the most trivial symptoms of stress. Dependence and tolerance occur after prolonged use. This has recently resulted in litigation brought by patients who have suffered from dependence on benzodiazepines.

Because chlordiazepoxide was not designed to be a benzodiazepine, certain features of its chemical structure were superfluous, notably the basic side chain and the $N$-oxide function. Simpler analogues were found, the first of these being synthesised in 1959 and marketed four years later as diazepam.\textsuperscript{47} It had more pronounced muscle relaxant properties than chlordiazepoxide and a half-life of one to two days as it was slowly cleared from the body.

Benzodiazepines were used for treating chronic anxiety states. Some of them, including diazepam, formed an active metabolite such as nordiazepam or something similar, which was responsible for their effects. Unfortunately, nordiazepam took from two to five days before being cleared from the body, hence its concentration gradually built up as more doses were taken. Recognition of this led to the introduction of benzodiazepines that did not form this type of active metabolite or which were rapidly eliminated. Examples of this include oxazepam,\textsuperscript{48} itself a metabolite of diazepam, and lorazepam.\textsuperscript{49} As these are alcohols, they are glucuronidated in the liver and quickly eliminated by the kidneys.

Flumazenil was synthesised in 1979.\textsuperscript{50} The replacement of a phenyl group by a carbonyl removed most of the typical sedative and anxiolytic activity, but as it still fitted the benzodiazepine receptor flumazenil acted as an antagonist when a moderate dose was
administered. It has been used to reverse the sedation produced by benzodiazepines, either when given as medication during short surgical procedures or in overdosage by drug abusers.

Seeking a product with which to enter the growing market in psychotropic drugs, the Tanabe Seiyaku Company of Japan prepared analogues of an antidepressant called thiazesim, in which a hydroxyl group or an O-acyl group was introduced at the 3-position of the benzothiazepine ring system. This manoeuvre had been instituted in the knowledge that the presence of a hydroxyl group in the equivalent position of diazepam had produced a more potent drug that did not accumulate in the body, e.g. oxazepam and lorazepam. When fully evaluated, these 3-substituted 1,5-benzothiazepines lacked sufficient novelty for them to be marketed. However, routine screening revealed that the 3-O-acyl benzothiazepines exerted a strong coronary vasodilator effect in the anaesthetised dog at dose levels that produced minimal central effects. Analogues were synthesised and it was established that introduction of a methyl or methoxy group at the 4-position of the benzene ring enhanced potency. Diltiazem was then found to have good oral absorption coupled with high efficacy and low toxicity. As it was a racemic mixture, its isomers were examined. As the dextro isomer possessed all the vasodilating activity, it was selected for clinical evaluation after it was fully evaluated by pharmacologists who demonstrated that it had a papaverine-like vasodilating action on the coronary artery and antagonised calcium ion flow across cardiac muscle membrane stores. The novelty of this action resulted in diltiazem being the first coronary vasodilator to be described as a ‘calcium antagonist’. The anti-arrhythmic activity also observed was due to the fact that there are fast and slow calcium channels, and diltiazem not only blocks calcium transport through the slow channels but also delays their recovery.

Diltiazem is now used in the treatment of angina and a slow-acting formulation is available for patients with hypertension who respond poorly to beta-blockers.

**Hypnotic Benzodiazepines**

Among the earliest chemical modifications effected on the benzodiazepine nucleus was the introduction of the nitro group, as this offered chemists an opportunity of subsequent structural variation. Several nitro compounds were prepared by Sternbach and his colleagues, of which nitrazepam proved to be much more potent than chlordiazepoxide in both mice and cats. Subsequent investigations showed that sleep could be induced by larger doses that were well below the toxic threshold. Indeed, so wide was the margin of safety that self-poisoning with nitrazepam was most unlikely to occur. This safety factor alone ensured worldwide acceptance of this new hypnotic. However, nitrazepam had a half-life of around 26 hours and so persisted in the body long enough to cause a hangover effect when patients awakened.
Temazepam was developed by Wyeth Laboratories in Radnor, Pennsylvania, and became a very popular hypnotic because there was no hangover effect.\textsuperscript{49} It was more susceptible than nitrazepam to metabolic deactivation in the liver, with a half-life of 8–10 hours, and no active metabolite formed. Unfortunately, temazepam was widely abused as an illicit recreational drug. Other short-acting hypnotics that were introduced include lormetazepam\textsuperscript{54} and loprazolam.\textsuperscript{55}

Midazolam was a short-acting anxiolytic agent developed by Hoffmann–LaRoche.\textsuperscript{56} Formulated as the hydrochloride salt, it is the only water-soluble benzodiazepine available for injection. When injected it has a half-life of about 1–3 hours and produced amnesia for a period of about 10 minutes after administration. It is also given continuously by the intravenous route in order to sedate patients undergoing intensive care.

Tricyclic Antidepressants

The recognition of the tranquillising properties of chlorpromazine in the mid-1950s led psychiatrists to test it and its analogues in a variety of clinical conditions. Roland Kuhn of the Cantonal Psychiatric Clinic, Munsterlingen, Switzerland, noticed that chlorpromazine produced effects that reminded him of those he had observed when testing an antihistamine that had been sent to him by Geigy for testing as a hypnotic. On that occasion, Kuhn had suggested further studies would be worth while, but this suggestion was ignored. This time, a long letter he wrote to Geigy was taken seriously, especially as the antihistamine had a striking structural resemblance to chlorpromazine. He received further samples of the antihistamine, code-named G22150. While it was soon found to have interesting properties, it had too many side effects. Geigy then sent Kuhn samples of imipramine, an analogue of G22150 with a side chain identical to that of chlorpromazine.\textsuperscript{57,58}

Kuhn thoroughly evaluated imipramine in a variety of psychiatric conditions. Early in 1956, it was administered to several patients suffering from endogenous depressions. After only three patients had been treated, it became clear that this new tricyclic compound had unique properties. A letter sent to Geigy at the beginning of February that year referred to the pronounced antidepressant activity of the new drug. At the Second International Congress of Psychiatry, held in Zurich 7 months later, an audience of a dozen people heard the first public disclosure of this major advance. A subsequent publication caught the attention of a wider audience.\textsuperscript{59,60} Since then, imipramine has been administered to millions of patients with impressive results.
Rival companies responded by introducing their own antidepressants, which had similar activity to imipramine. For example, trimipramine was synthesised as an analogue of trimeprazine at the Rhône–Poulenc laboratories, while clomipramine was introduced by Smith, Kline and French.

The recognition of the antidepressant action of imipramine revealed that minor structural alterations in the central ring of phenothiazine tranquillisers could radically change their pharmacological profile. This stimulated medicinal chemists to synthesise novel tricyclic compounds. As has been seen, replacement of the nitrogen atom in the central ring of chlorpromazine led to the introduction of the thioxanthenes as tranquillisers in 1958. Similarly, replacement of the sulfur atom in the thioxanthene system resulted in the first of the dibenzocycloheptadienes, namely amitriptyline, which was synthesised by several groups in 1960. One of the first was Merck Sharp & Dohme Research Laboratories, who also prepared nortriptyline. Both resembled imipramine insofar as they were antidepressants rather than tranquillisers, but were noticeably less stimulating. This has made them more suitable than imipramine for treating agitated, anxious patients who were also depressed.

Analogues of amitriptyline include doxepin, in which a carbon atom in the central ring is replaced by oxygen. It has a similar clinical profile to amitriptyline, but may be somewhat less cardiotoxic in overdosage. Dosulepin is similar in its activity.
In 1958, researchers at the Wander Research Institute in Basle synthesised analogues of imipramine in which one or more heteroatoms replaced carbon atoms in the central ring. Particularly interesting from a pharmacological point of view were several amidines, including the antidepressants amoxapine and clozapine. Amoxapine had very similar antidepressant properties to imipramine, but clozapine proved to be an antipsychotic drug. However, it was atypical insofar as it did not produce extrapyramidal side effects and was of value in patients who had failed to respond to treatment with other antipsychotic drugs. It was marketed in Switzerland and Austria in 1972, but a high incidence of agranulocytosis was observed during a clinical trial in Finland three years later. This resulted in the use of clozapine being severely restricted. However, in 1988 a major study by John Kane of Hillside Hospital in Glen Oaks, New York, revealed the outstanding activity of clozapine in the treatment of schizophrenics who had not responded to therapy with conventional antipsychotic drugs. It became more frequently prescribed thereafter.

When Lilly researchers examined the effect of replacing either of the benzene rings in clozapine with a thiophen ring, they found four compounds worthy of further study. Only one of these proved to be safe enough for human studies. It was marketed under the name ‘olanzapine’ in 1996 as a safer alternative to clozapine. Quetiapine is a similar atypical antipsychotic drug.

Carbamazepine was synthesised by Walter Schindler at the Geigy laboratories in Basle in 1953 when the company was investigating analogues of chlorpromazine. It was only some years later that its anticonvulsant properties were recognised. The first clinical study was not carried out until 1963, and it seems to have taken longer than most anticonvulsants to become established in clinical practice. Carbamazepine is now considered to be as effective as phenytoin in the control of partial and tonic–clonic seizures.

Selective Serotonin Reuptake Inhibitors

During the 1960s, Swiss psychiatrist Paul Kielholz differentiated tricyclic antidepressants for clinical application on the basis of whether they possessed the ability to sedate, stimulate drive or improve the mood of patients. At that time there was a broad consensus that the tricyclic antidepressants acted by inhibiting the reuptake of norepinephrine back into the neurones from which it was released, thereby elevating the level of the hormone. By the end of the decade, however, there was mounting evidence that the reuptake of 5-HT was also blocked. The first to associate the thinking of Kielholz with the biochemical advances were the Russians Izyaslav Lapin and Gregory Oxenkrug of Bekhterev’s Psychoneurological Research Institute in Leningrad. In 1969, they suggested that increased serotonergic activity in the brain involving tryptophan and its metabolites, including 5-HT, accounted for the mood-elevation effect of antidepressants, while increased noradrenergic activity was responsible for the motor
and energising effects. Several groups of researchers followed this up by seeking selective serotonin reuptake inhibitors (SSRIs).

At the Karolinska Institute, Arvid Carlsson examined the effects of antihistamines on both 5-HT and norepinephrine uptake in tissues. Although most had mixed activity, diphenhydramine affected only 5-HT uptake. In collaboration with Peder Berntsson and Hans Corrodi of Aktiebolaget Hassle, based in Gothenburg and part of Astra, Carlsson quickly developed a pheniramine analogue as a potent SSRI in the spring of 1971. Several years later it was marketed in Europe as zimelidine in 1982, but was withdrawn by Astra the following year because ten cases of the Guillaine–Barré syndrome had been reported out of 200,000 prescriptions. This neurological disorder was characterised by progressive muscular weakness. Fortunately, a slow recovery over a period of months occurred in all patients once medication had ceased.

The second SSRI to be marketed in Europe also had to be withdrawn shortly after its introduction, this time because it produced agranulocytopenia in a few patients. The drug was an antihistamine analogue called indalpine, developed by Gerard Le Fur and his colleagues at Fournier Frères, a company that became part of Rhône–Poulenc.

The next SSRI to be introduced was fluvoxamine. It remained on the market without encountering the problems faced by its predecessors. However, during early trials concern was expressed over the number of patients who committed suicide before the drug had exerted its beneficial action. Similar concerns have been raised about other SSRIs, despite their main advantage over tricyclic antidepressants being their enhanced safety margin when deliberate overdoses are consumed. This issue is highly contentious and is being examined in the law courts.

Several other SSRIs were subsequently marketed, including one that has become a household name. Bryan Molloy of Eli Lilly made analogues of diphenhydramine for Robert Rathbun and Richard Kattau to screen for inhibition of norepinephrine and 5-HT uptake.
They found $N$-methyl-phenoxyphenylpropylamine to be twice as potent at inhibiting uptake of 5-HT as it was at inhibiting norepinephrine uptake, so a series of analogues of it were synthesised. This resulted in the discovery of fluoxetine as an SSRI. Eli Lilly marketed it in 1988. Since then, it has become the most frequently prescribed antidepressant drug. It was especially popular in the United States under its proprietary name of Prozac®.

**ANTIFIBRINOLYTIC DRUGS**

S. Okamoto set up a screening programme to find antifibrinolytic drugs that could be used to stop haemorrhage. Among the 400 or so compounds he tested were basic amino acids that were found to have some activity, the most effective being lysine.

Since lysine had inadequate potency for clinical applications, analogues of it were examined. This led to the discovery in 1957 that removal of the $\alpha$-amino group greatly enhanced activity, $\varepsilon$-aminocaproic acid being ten times as potent as lysine. Further investigation showed that it was an inhibitor of plasminogen activation. It was introduced into the clinic as a haemostatic agent, but was superseded by tranexamic acid.

When Okamoto and his colleagues screened a large number of analogues of $\varepsilon$-aminocaproic acid they discovered that the distance between the carboxylic and amino groups was critical, as was the nature of the linkage between them. After finding that a benzene ring could be used as a linkage, a cyclohexane ring was shown to be even more potent. The most potent compound was tranexamic acid, so named because it was a trans isomer. The cis isomer was inactive as the amino and carboxylic groups were positioned too close to each other. Tranexamic was introduced to stop haemorrhage during surgery.

**NON-STEROIDAL ANTI-INFLAMMATORY DRUGS**

During the 1950s, it became widely recognised that the long-term use of corticosteroids in rheumatoid arthritis caused serious problems that were inherent in the nature of the medication. In 1955, Stewart Adams at the Boots Pure Drug Company laboratories in Nottingham established a screen to find a safe, orally active anti-inflammatory agent. This involved ultraviolet (UV) irradiation of the backs of guinea pigs 30 minutes after they had received a test compound by mouth. Adams had established that this procedure reliably indicated the ability of aspirin to reduce inflammation and could be used to test alternatives to it in the search for a more potent compound with fewer side effects.

The chemist working with Adams was John Nicholson. Both were convinced that the presence of a carboxylic acid group was responsible for the anti-inflammatory activity of aspirin and some of its analogues. Adams screened phenylacetic and phenoxyacetic acids previously made by the company as potential herbicides. After 2-(4'-ethylphenoxy)propionic acid proved to be several times as potent as aspirin, more than 200 aryloxyalkanoic acids were synthesised and tested. Eventually, 2-(4'-phenylphenoxy)propionic acid emerged as a candidate for clinical investigation. Its ethyl ester was preferred since it was expected to cause less gastric irritancy. However, when put on clinical trial in 1960 it turned out to be
inactive in patients with rheumatoid arthritis. Significantly, its analgesic and antipyretic effects were feeble. This led to a decision that all active test compounds should in future be tested for analgesic and antipyretic as well as anti-inflammatory activity.

![Chemical structures](image)

Attention was now switched to 4-alkylphenyl and biphenylalkanoic acids. Approximately 450 more compounds were synthesised and screened. Two of these were examined in the clinic, but produced rashes in patients. Finally, ibufenac was found to exhibit more than four times the potency of aspirin. After an early clinic trial gave encouraging results, it was marketed in 1966. However, it had to be withdrawn soon after when evidence of an unacceptable incidence of jaundice appeared.

Ibuprofen was selected as an acceptable alternative to ibufenac after animal studies confirmed that it did not accumulate in the liver or produce ulcers in dogs, as had some similar compounds. It proved to be a safe, effective anti-inflammatory agent with analgesic and antipyretic properties and was marketed in 1969. Ibuprofen became widely prescribed throughout the world in the wake of increasing concern about the hazard of gastric bleeding caused by aspirin. Such was its relative safety that in 1983 it became available in the United Kingdom as a non-prescription analgesic on account of its having the lowest overall rate of reporting of suspected adverse reactions among the non-steroidal anti-inflammatory agents, some 20 million prescriptions having been issued over the preceding 15 years.

Among the 600 compounds screened by Adams before the introduction of ibuprofen were several 4-biphenylalkanoic acids. These proved to be highly potent anti-inflammatory agents, but were abandoned in favour of the less-potent phenylalkanoic acids that were at that time believed to be less toxic. After the introduction of ibuprofen these acids were again investigated, resulting in the introduction of flurbiprofen. Although it turned out to be 5–10 times as potent as ibuprofen, this did not confer any significant therapeutic advantage. The indications for its use are identical to those for ibuprofen.
Rival manufacturers quickly developed analogues of ibuprofen. Syntex introduced naproxen, which was twice as potent as ibuprofen and had a longer duration of action, allowing twice-daily dosing. Beecham Pharmaceuticals developed the related nabumetone as a prodrug that rapidly underwent metabolic activation in the liver to form the acid. Their intention had been to minimise the inhibition of prostaglandin synthesis in the stomach and so reduce gastric irritation, but this would only have been relevant if that inhibition had been a local rather than a systemic effect. Rhône-Poulenc marketed ketoprofen, which was about ten times as potent as ibuprofen and had a longer duration of action. Fenoprofen was introduced by Lilly. It had a similar duration of action to ibuprofen but was 2–3 times as potent.

A number of other non-steroidal anti-inflammatory acids were marketed in the 1970s and 1980s. Among them was benoxaprofen, which was developed at the Lilly Research Centre in Surrey, England. When it was launched in the United Kingdom in March 1980, benoxaprofen was promoted as an anti-arthritis agent that could be taken as a single daily dose because of its resistance to metabolic degradation. At the time, this was considered helpful in ensuring good patient compliance. There were also anecdotal reports of dramatic improvements in the condition of seriously crippled patients.

In February 1982, Hugh Taggart at Queen’s University in Belfast reported the deaths of five elderly patients who had received benoxaprofen. Urgent enquiries ensued and the UK Committee on Safety of Medicines withdrew the Product Licence in August of that year, amid intense media coverage. By that time, 83 fatalities had occurred among three-quarters of a million patients in the United Kingdom who had taken the drug. Most had died from renal or hepatic failure.

The 15th International Congress of Rheumatology held in Paris in June 1981 had been told that benoxaprofen was slowly excreted, its biological half-life being as long as four days in elderly patients. Earlier reports had indicated that the half-life was 33 hours in humans, justifying the convenience of once-daily dosage. The implications of a more prolonged half-life in elderly patients had not then been obvious. It is with the benefit of hindsight that they certainly are now. The issue relates to the question of toxicity, for it is only if a drug is relatively toxic that a problem arises from its accumulation in patients with poor renal function. Although reports of photosensitivity and nail damage had been received, benoxaprofen was not at that time thought to be any more toxic than other non-steroidal anti-inflammatory agents.

The outcome of this tragic affair would have been different if more information about the effects of benoxaprofen in elderly people had been available. The manufacturer had mentioned the need for dosage reduction in a pamphlet issued three months after the Paris Symposium, but this seems to have been generally overlooked. Only 52 patients over the age of 65 had received the drug in clinical trials, but this was not exceptional since it was not expected that elderly patients should be recruited specifically for such trials. Lessons were learned from this affair, not least being the importance of an exemplary level of vigilance required from both manufacturers and licensing authorities. Furthermore, there is now recognition that elderly patients should not be prescribed long-acting drugs when alternatives are available.
Selective COX-2 Inhibitors

In 1991, Dan Simmons and his colleagues at Brigham Young University in Utah discovered that there was a second type of cyclooxygenase enzyme inhibited by aspirin and the non-steroidal anti-inflammatory agents. This COX-2 was principally involved in producing prostaglandins during inflammation, whereas COX-1 was involved in routine physiological processes such as platelet aggregation. Pharmaceutical companies immediately recognised the implications and began seeking selective COX-2 inhibitors that would be free from the side effects of existing anti-inflammatory drugs that all lacked selectivity.

G.D. Searle launched a screening programme that was to test over 2500 compounds. These were at first screened against cloned COX-2 enzyme, but it was found that an assay in rodents was more reliable. Around 10% of the compounds were selected for further screening, from which seven emerged as potential drug candidates and were examined in several species of animals. A compound from the company’s agrochemical library of compounds turned out to be both a selective COX-2 inhibitor and an anti-inflammatory agent. It was marketed in 1999 with the approved name of 'celecoxib'. It was hoped that it would produce a lower incidence of side effects than other anti-inflammatory drugs.

Rival companies introduced several more selective COX-2 inhibitors during the next few years. One of these, rofecoxib, was suddenly withdrawn in 2004 after Merck had conducted a study that revealed that patients taking their product faced a higher risk of heart attacks and stroke than those on a placebo.

ETHAMBUTOL

In the course of an extensive screening programme, researchers at Lederle Laboratories discovered that \(N,N'\)-diisopropylethylenediamine had antituberculosis activity comparable with that of isoniazid. The sole drawback was its greater toxicity.

An extensive series of analogues was synthesised, culminating in the development of ethambutol, which was reported in 1961 to be a particularly promising drug. This early promise was fulfilled and ethambutol is still used in combination with other agents. The main problem with it is that visual side effects occur and thus patients require to be regularly monitored while receiving treatment.

LEVAMISOLE

The Janssen Research Laboratory at Beerse in Belgium initiated an extensive screening programme in which 2721 novel heterocyclic compounds were tested for anthelmintic activity
against three types of parasitic worms before an aminothiazole derivative, R6438, was found to be effective in chickens and sheep.

![Chemical structures of R6438, R8141, and levamisole]

Its failure in mice and rats pointed to the possibility that it had to undergo metabolic conversion to an active drug that was only formed in some animals. All the metabolites were then isolated and synthesised. R8141 was the only active one, but was difficult to produce and, in addition, was unstable in water. A large series of its analogues was synthesised, of which one met all the requirements for possible clinical application. This was given the approved name of ‘tetramisole’, but it was its laevo isomer that was selected for medicinal use since it was several times more potent, yet no more toxic. It is employed under the name ‘levamisole’ as an ascaricide to eliminate the common roundworm.

**PYRANTEL**

In the mid-1950s, Pfizer researchers at Groton, Connecticut, established a screening programme to find new anthelmintic agents. In order to widen the score of the screens, laboratory mice were inoculated with three different organisms, namely the tapeworm *Hymenolepis nana*, the nematode *Nematospiroides dubius* and the pinworm *Syphacia obvelata*. Out of a large number of compounds submitted for screening, only compound I emerged with any evidence of activity. When administered by mouth to sheep, it had little activity, probably because it hydrolysed to the compounds from which it had been synthesised, namely 2-thenylthiol and 2-imidazolidone.

![Chemical structures of compound I, pyrantel, and oxantel]

Analogues of compound I designed to resist hydrolysis were synthesised at the Pfizer research centre in Sandwich, England. An early advance came when a methylene group replaced the sulfur atom in the bridge between the two rings to give a compound that was active against a variety of roundworms that infested sheep. As this compound was toxic, analogues were synthesised. Optimal activity against a variety of nematodes was obtained by enlarging the imidazoline to a tetrahydropyrimidine ring, as in pyrantel. This was an orally active broad-spectrum anthelmintic that was effective against roundworms, hookworms and threadworms in both humans and animals.

Oxantel was one of several analogues of pyrantel that were prepared in order to examine the relationship between aromatic ring substitution and anthelmintic potency. It had only one-tenth of the activity of pyrantel in the mouse screen against *N. dubius*, but was active against the tapeworm *H. nana*, unlike pyrantel. When tested in dogs with whipworm infestation, it was also active. This activity against whipworm infection compensated for its narrow spectrum of anthelmintic activity, and became the principal clinical application.

**NIFEDIPINE**

Because 1,4-dihydropyridines played an important role in biochemical processes yet had never been investigated pharmacologically, the medicinal chemistry group at the Bayer laboratories...
submitted a variety of these for screening. After some of these compounds were found to exhibit a measurable effect on cardiac output, more than 2000 analogues were synthesised and screened.\textsuperscript{102} In 1967, Friedrich Bossert and Wulf Vater applied for a South African patent in which they claimed that nifedipine possessed marked coronary vasodilating activity.\textsuperscript{103} Further investigations revealed that nifedipine selectively blocked calcium channels in the conductive cells of the heart and vascular smooth muscle, thereby inhibiting the entry of ionised calcium and its release from intracellular stores. Since calcium was required for membrane depolarisation and muscle contraction, nifedipine relaxed smooth muscle both in the myocardium and in the walls of blood vessels. The outcome of this was dilation of the coronary vessels and a fall in vascular resistance, with a consequent reduction of cardiac afterload, work and oxygen consumption. As this was of major importance in the treatment of vascular disorders such as angina and hypertension, nifedipine was marketed in 1975.

Several analogues of nifedipine have been developed which have a longer duration of action and hence are less likely to cause fluctuations in blood pressure and reflex tachycardia, e.g. felodipine\textsuperscript{104} and amlodipine.\textsuperscript{105} Nimodipine had a high specificity for calcium channels in cerebral blood vessels and hence was able to increase cerebral blood flow without decreasing blood pressure.\textsuperscript{106} This made it valuable in the prevention of cerebral arterial spasm after subarachnoid haemorrhage.

**CARMUSTINE**

In 1955, following a decade of remarkable progress in the sphere of cancer chemotherapy in the United States, Congress allocated large sums of money to set up a screening programme run by the Cancer Chemotherapy National Service Center (CCNSC), which was part of the National Cancer Institute. The first contracts were awarded to four screening centres that confidentially tested large numbers of compounds submitted by academic and industrial researchers. By the end of the decade, these centres were testing around a thousand chemicals each month against animal tumours.

In 1959, researchers at the Wisconsin Alumni Research Foundation discovered that 1-methyl-1-nitroso-3-nitroguanidine (MNNG), an intermediate used by organic chemists to prepare the unstable alkylating agent diazomethane, had antileukaemic activity in mice. Unfortunately, human trials were disappointing. Nevertheless, when Thomas Johnston, George McCaleb and John Montgomery at the Southern Research Institute in Birmingham, Alabama, were notified by the CCNSC of the activity of MNNG, they immediately began an evaluation of related compounds as there was considerable concern that half of the long-term survivors among children who received combination chemotherapy for acute leukaemia were
dying from meningeal leukaemia. They found that an alternative compound used in the
synthesis of diazomethane, namely 1-methyl-1-nitrosourea (MNU), was more active than
MNNG. As it was also more lipophilic, they believed it would penetrate the central nervous
system and so be effective in meningeal leukaemia. After preliminary tests in animals injected
intracerebrally with leukaemic cells, MNU was investigated further by the National Cancer
Institute.107 While this was taking place, analogues of MNU were synthesised and tested at the
Southern Research Institute. The researchers there believed that the antileukaemic activity was
due to MNU decomposing into diazomethane hydroxide, a powerful alkylating agent.
Attempts were therefore made to find a more active biological alkylating agent by replacing
the N-methyl group with similar groups that would produce diazoalkyl hydroxides with
different activity profiles. This led to the synthesis of carmustine.108 When it was tested in mice
injected with L-1210 leukaemic cells, not only was it the most active of 23 compounds
submitted for evaluation and far superior to MNU but it was also the first to cure such
mice.109 An early clinical trial confirmed the value of carmustine.110

As expected, carmustine penetrated into the central nervous system because of its high lipid
solubility. It controlled meningeal leukaemia in some children, but as this soon became
preventable by irradiating the cranium at the outset of acute lymphocytic leukaemia
treatment, this application of carmustine became redundant. It is instead used to treat brain
tumours, advanced Hodgkin’s disease, lymphomas and myelomas. Because of its alkylating
activity, local tissue damage would be caused if it were taken by mouth or injected
intramuscularly. Hence carmustine is given intravenously so that immediate dilution by blood
occurs. Lomustine, which was also developed at the Southern Research Institute, can be taken
by mouth.111

PRAZIQUANTEL

In 1972, a screening programme at the Bayer Institute for Chemotherapy in Wuppertal
revealed anthelmintic activity in novel pyrazinoisoquinolines that had been synthesised in the
laboratories of E. Merck and Company of Darmstadt in the course of a joint project between
these two German companies. Praziquantel was selected from over 400 compounds for further
investigation. It exhibited outstanding efficacy against all known intestinal cestode infections
in humans, as well as a great many in animals.112

Further studies revealed not only that a single oral dose was capable of eradicating these
infections but also that praziquantel was highly effective against schistosomiasis and a variety
of other parasitic infections.113 It became the first drug to receive World Health Organization
approval for use in mass eradication programmes aimed at eliminating a broad range of parasitic infections.

**MILRINONE**

At the Sterling–Winthrop Research Institute in Rensselaer, New York, a screening programme was established in order to find inotropic compounds with cardiotonic activity similar to that of the cardiac glycosides. Amrinone was discovered to be among the most active of the compounds capable of increasing the contractility of cardiac muscle. In addition, it has a useful vasodilating action. These properties were found to be due to its ability to act as a selective phosphodiesterase inhibitor in cardiac and vascular muscle, raising intracellular levels of cAMP. This resulted in an increase in calcium levels, which accounted for the increased force of cardiac contraction. As it had little effect on heart rate, amrinone was suitable for use as a cardiac muscle stimulant in congestive heart failure. However, the Sterling–Winthrop researchers found milrinone to be many times more potent, so it was preferred for clinical use.

Several other companies sought selective phosphodiesterase inhibitors, enoximone being developed at the Merrell Dow Research Center in Cincinnati after it was discovered that imidazole had some activity. It was less potent than milrinone.

**NAFTIFINE**

Naftifine was found to be an antifungal agent during screening at the Sandoz Research Institute in Vienna. It was shown to have a novel mode of action which involved blocking the synthesis of ergosterol by inhibiting squalene oxidase, but it was only suitable for topical use.

Over a thousand analogues of naftifine were prepared in order to establish the requirements for optimal antifungal activity. From this, acetylenic allylamines were selected for special attention, resulting in the development of terbinafine as an orally active antifungal agent with significantly greater activity than naftifine.

**PROPOFOL**

At the Alderley Edge laboratories of ICI (now AstraZeneca), screening in mice for potential anaesthetics revealed the anaesthetic activity of 2,6-diethylphenol.
Roger James and J.B. Glen then prepared and examined further alkyl-substituted sterically hindered phenols in order to optimise activity in this series. They found that it was necessary to strike a balance between the minimum steric hindrance providing adequate potency and excessive steric crowding, which caused loss of anaesthetic activity. In addition, lipophilicity had to be limited in order to avoid slower kinetics through binding to plasma proteins. The most active compounds were di-sec-alkyl substituted and had 6 to 8 carbon atoms in the side chains. Propofol was the only compound among those studied that emerged with a satisfactory profile when evaluated as an intravenous anaesthetic. It is now widely used.

**ANGIOTENSIN II ANTAGONISTS**

Angiotensin II is a powerful hypertensive agent because it binds to receptors known as AT$_1$ and AT$_2$ to cause vasoconstriction. Using these receptors as a target for high throughput screening (HTS), the Japanese company Takeda tested a vast number of compounds and obtained a ‘hit’. Analogues of this were then prepared by DuPont chemists in order to find a compound that was selective for the AT$_1$ receptor. This was given the approved name of ‘losartan’ and was introduced into the clinic in 1993.

Unlike the ACE inhibitors, losartan and its analogues do not cause the breakdown of bradykinin, hence patients do not experience the unwelcome dry cough caused by ACE inhibitors. In general, the clinical value of angiotensin II antagonists matches that of the ACE inhibitors.
Several other angiotensin II antagonists have been introduced, including eprosartan,124 candesartan,125 irbesartan,126 valsartan,127 olmesartan128 and telmisartan.129 Even a superficial glance at their chemical structures shows that they are derived from either losartan or the lead compound from HTS that led to its development. This reveals the pattern of drug development by rival companies that will be seen when future drugs derived from HTS are introduced.

**IMATINIB**

In 1973 at the University of Chicago, Janet Rowley established that the missing portion of chromosome 22, the ‘Philadelphia chromosome’, had translocated to chromosome 9 in patients with chronic myelogenous leukaemia (CML). During the next decade it was confirmed that one gene from each chromosome (Bcr in chromosome 22, Abl in chromosome 9) fused to form a new gene, designated Bcr–Abl. This gene produced the rogue protein that caused CML. David Baltimore and his colleagues at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported that this protein was a tyrosine kinase enzyme. In the early 1990s, the Swiss company Ciba-Geigy became interested in the possibility of designing an inhibitor of the enzyme. Using high throughput screening of the company’s small molecule libraries, a 2-phenylaminopyrimidine emerged as a weak inhibitor of several protein kinases that could serve as a lead for further development. Introduction of a methyl at the 6-position and of a benzamide on the phenyl ring enhanced inhibitory activity towards Abl. A promising inhibitor ultimately emerged from this work, but it lacked water solubility and oral bioavailability was poor. These difficulties were overcome by attaching an N-methylpiperazine to form imatinib.130

![imatinib structure](image)

Imatinib appears to be giving encouraging results as a highly selective chemotherapeutic agent for the treatment of CML.131 Among untreated patients who receive imatinib, around 60% respond to treatment and experience few side effects. There have been no long-term studies yet, but it is clear that this is the first drug that can treat cancer by targeting a protein that causes the disease. Several companies are currently investigating other protein kinase inhibitors.

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Horace Walpole introduced the word ‘serendipity’ over 200 years ago in a letter he wrote to a friend after reading a poem about three princes of Serendip (Sri Lanka) who had repeatedly made discoveries they were not seeking. Walpole explained that his new word referred to chance discoveries that were exploited with sagacity. It is a word that repeatedly reappears in the literature of drug research, not so much with regard to the discovery of new drug prototypes but rather with regard to discovering new applications of existing drugs and their analogues. This chapter and the next, however, deal with serendipitous discovery of several prototypes and the drugs that evolved from them.

Some drug prototypes have been discovered as a consequence of their effects being noticed by those exposed to them. While the actions of certain chemicals on the nervous system were obvious, their relevance to clinical practice could easily be overlooked unless an individual had the sagacity to exploit them. This has also been the case in the laboratory, when experiments sometimes resulted in unexpected outcomes, the most celebrated instance being the discovery by Fleming of penicillin.

**NITRATES**

The product generally known as amyl nitrite actually consists mainly of isoamyl nitrite. It was first synthesised at the Sorbonne in 1844 by Antoine Balard, who reported that its vapour had given him a severe headache. Frederick Guthrie of Owens’ College, in Manchester, experimented with amyl nitrite and found that its most significant action was upon the heart. He found that it produced intense throbbing of the carotid artery, flushing of the face and an increase in heart rate.

Thomas Lauder Brunton, a newly qualified house surgeon at the Edinburgh Royal Infirmary, pioneered the clinical application of the sphygmograph in 1867 by employing it to monitor the rise in blood pressure that accompanied attacks of angina pectoris in his patients. Faced with one particular patient who nightly experienced paroxysmal attacks of angina, he followed the fashion of the time by removing blood through either cupping or venesection. This appeared helpful, leaving Brunton convinced that the relief of pain was due to the lowering of arterial pressure. He then speculated that amyl nitrite might help his patient, having seen his friend Arthur Gamgee use it in animals to lower blood pressure.
Brunton obtained a sample of amyl nitrite from Gamgee and poured some on to a cloth for his patients to inhale. Within a minute, their agonising chest pains had disappeared, several remaining free of pain for hours. The success of the drug was assured, and it was universally adopted after Brunton reported his observations.\textsuperscript{3,4}

Over the next few years, Brunton established that other nitrites had similar effects. He also examined nitroglycerin, which had become readily available following Alfred Nobel’s discovery of its value as an explosive. Finding that when he and a colleague tried nitroglycerin it gave them both a severe headache, Brunton did not pursue its use further. His observations were mentioned in the \textit{St Bartholomew’s Reports} in 1876, but matters did not rest there as William Murrell at the Westminster Hospital decided to resolve the conflicting reports in the literature over whether or not nitroglycerin caused severe headache. Its discoverer, the Turin chemist Ascanio Sobrero,\textsuperscript{5} had reported in 1847 that he experienced an intense headache after merely tasting a drop of it placed on his finger. Others had a similar experience, including Arthur Field, a Brighton dentist, who claimed that he had alleviated toothache and neuralgia by applying a drop or two of a dilute alcoholic solution of nitroglycerin to the tongue.\textsuperscript{6} Were it not for the ensuing headache, so he claimed, nitroglycerin could be a valuable remedy. In an ensuing correspondence, some writers confirmed Field’s observations, while others claimed nitroglycerin produced no effects whatsoever despite their swallowing large amounts. Murrell suspected that this confusion could have arisen from variation in the susceptibility of individuals to nitroglycerin.

In the course of one of his clinics, Murrell casually licked the moist cork of a bottle of nitroglycerin solution that was in his pocket. Within a few moments he began to experience throbbing in the neck and head, accompanied by pounding of his heart, the very effects that Field had so vividly described. Such was his discomfiture that Murrell could not continue with his examination of a patient. After five minutes, he had recovered sufficiently to resume his duties, but his severe headache lasted all afternoon. He subsequently tested nitroglycerin on himself on a further 30 or 40 occasions before persuading friends and volunteers to take part in a trial of its effects. This brought home to him the similarity between its action and that of amyl nitrite, but there was one important difference. Charting the changes in blood pressure of the volunteers, Murrell established that although it took 2 or 3 minutes for nitroglycerin to produce its effects, as opposed to only 10 seconds or so for amyl nitrite, they persisted for about half an hour. This was in marked contrast to amyl nitrite, the effects of which wore off after 5 minutes. This persuaded Murrell that nitroglycerin might be superior in the treatment of angina pectoris. He also confirmed that the headache was due to overdosage. The correct dose turned out to be in the order of 0.5–1 mg when the drug was formulated in tablets that were allowed to dissolve slowly under the tongue, whereas around 100–300 mg of amyl nitrite had to be inhaled to produce similar effects. If the nitroglycerin were swallowed, a larger dose was required since absorption from the gut was less efficient than from the mouth, or even through the skin.

Murrell began treating patients with nitroglycerin in 1878, and the following year published his first report in the \textit{Lancet}.\textsuperscript{7} This led to the general adoption of the drug into clinical practice. British doctors took steps to avoid any unnecessary alarm that might result if patients discovered they were receiving the same explosive as was in dynamite by renaming it as glyceryl trinitrate or trinitrin.

Tablets of glyceryl trinitrate taken sublingually are still commonly used to deal with attacks of angina caused by exertion, but any prophylactic effect is of short duration. In recent years, an old form of percutaneous treatment with ointment containing glyceryl trinitrate has been adapted by the use of much more reliable transdermal patches that release the active constituent in a controlled manner over a prolonged period during which there is effective prophylaxis against anginal attacks.

As nitroglycerin was an ester prepared by the nitration of a polyhydroxylic alcohol, analogues of it were synthesised by the nitration of sugars in attempts to obtain longer-acting...
drugs for prophylactic use. The first was pentaerythritol tetranitrate, which was introduced in 1896, although its synthesis was not reported until 1901. There has been controversy as to whether it and other longer-acting nitrate esters such as isosorbide dinitrate are of any real value in preventing attacks of angina as the rate of their metabolism in the liver is such that little unchanged drug enters the circulation.

The active metabolite of isosorbide dinitrate is the mononitrate, which is twice as potent and has a less complicated pharmacokinetic profile that makes the response more predictable.8

LIDOCAINE

In the course of a pioneering chemical plant taxonomy study on a chlorophyll-deficient mutant of barley, Hans von Euler at the University of Stockholm isolated an alkaloid called ‘gramine’.9 In order to confirm that it was 2-(dimethylaminoethyl)-indole, von Euler’s assistant Holger Erdtman synthesised this compound in 1935. The synthetic product turned out to be an isomer of gramine and so was named ‘isogramine’.

On tasting a trace of isogramine, Erdtman noted that it numbed his tongue. Further investigation revealed that a similar local anaesthetic activity existed in its open-chain synthetic precursor. This persuaded Erdtman and his research student Nils Löfgren to seek less-irritant analogues for possible clinical use. The task proved daunting, as most of the active analogues were irritant. Löfgren’s persistence finally paid off after 57 compounds had been synthesised over a 7 year period. After his colleague Bengt Lundqvist had tested compound LL 30 on himself, he suggested it should be pharmacologically evaluated at the Karolinska Institute. The results were encouraging and clinical trials were then arranged. Löfgren then approached a small Swedish company called Astra, which resulted in lidocaine being marketed in Sweden in 1948.10 Over the next few years, as a result of its rapid onset of action, relative safety and freedom from irritancy, it attained a pre-eminence over all other local anaesthetics. It was also administered intravenously as an anti-arrhythmic agent in coronary care units, being preferred to procaine as it had a faster onset of activity. In 1960, Löfgren synthesised prilocaine, which had a wider safety margin.11

In 1957, scientists from the Swedish pharmaceutical company AB Bofors investigated the pharmacological activity of a series of lidocaine analogues in which the side chain had been partially incorporated into a cyclic system.12 This had been done to establish whether a cyclic
analogue offered any advantages. One of the resulting compounds, bupivacaine, turned out to be longer-acting, producing nerve blocks for up to 8 hours. It became widely used for continuous epidural anaesthesia during childbirth.

ANTICHOLINESTERASES

At the University of Berlin, in 1932, Willy Lange and his student Gerda von Kreuger took advantage of the recent ready availability of fluorine to prepare the first phosphorus–fluorine compounds. In the course of their work they experienced marked pressure in the larynx, followed by breathlessness, clouding of consciousness and blurring of vision. These effects were similar to those produced by nicotine, which was used as an insecticide, killing insects by relentlessly mimicking acetylcholine to disrupt nervous transmission. Lange mentioned this at the end of his paper. He left Germany shortly after.

At the Leverkusen laboratories of I.G. Farbenindustrie, Gerhard Schrader followed up this observation and prepared more than 2000 organophosphorus compounds as potential insecticides. It was shown (although not divulged) by Eberhard Gross at the company’s Elberfeld laboratories that these were anticholinesterases, some of which were highly poisonous to laboratory animals and thus of little value as insecticides. Unlike the natural anticholinesterase physostigmine, these organophosphorus compounds formed such a strong bond with the enzymes that their action was permanent. Under these circumstances, acetylcholine could not be broken down and nervous transmission was disrupted. This inevitably resulted in death.

The lethal action of the organophosphorus compounds was ruthlessly exploited in the development of war gases with the terrifying potential to kill entire populations. Their value as chemical warfare agents was fully appreciated by the German authorities, who stockpiled them for military use. The first agent to be so employed was tabun, synthesised by Schrader in 1936. Described as a ‘war gas’, tabun was actually a liquid that could be deployed in a fine dispersion. A plant disguised as a soap-making factory was opened at Dyhernfurth-am-Oder, near Breslau, near the Polish border in 1942 for its production. By the end of the war, 12 000 tons had been manufactured, and field forces were equipped with tabun-filled shells. Tabun was allegedly used for the first time in 1980 during the Iran–Iraq war.

The far more toxic sarin was developed by Schrader and Otto Ambros in 1938, being named after them and Rudiger and van der Linde of the chemical warfare division of the Wehrmacht; a mere 1 mg of this was capable of killing an adult within minutes after being absorbed through the skin. In the early 1950s, the United States supplied its chemical warfare units around the world with sarin, listed under the code name of GB. Later in the decade, production began of the even more toxic VX agent.

The British Ministry of Supply arranged for the potential of the fluorophosphonates to be studied at the physiology laboratory in Cambridge University by Edgar Adrian and his colleagues. They found, in 1941, dyflos to be the most toxic of the compounds originally prepared by Lange and von Krueger. Its prolonged miotic action on the eye convinced them that it was an anticholinesterase, and direct evidence for this was obtained. When the war ended, dyflos found occasional clinical application in the treatment of glaucoma.

American Cyanamid introduced malathion as an insecticide in 1951. It represented a major advance from the point of view of safety for both those who handled insecticides and...
also farm animals as it incorporated two features that reduced mammalian toxicity. Firstly, it was metabolised in insects to form the active insecticide malaoxon. The second inherent safety feature was that if it was absorbed it rapidly underwent esterase hydrolysis to form an acid that was rapidly excreted. This did not happen in insects, hence the enhanced safety of malathion which permits its topical application to treat scabies, head lice and crab lice.

![Chemical structures of malathion, metrifonate, and dichlorvos](image)

In 1952, Schrader and his colleagues at the Bayer laboratories in Elberfeld synthesised metrifonate. It had a sufficiently wide margin of safety for it to be administered as a systemic insecticide in domestic animals. Though itself not an anticholinesterase, metrifonate spontaneously rearranged in aqueous solution to form the active agent dichlorvos.

Jacques Cerf, a physician working in the Belgian Congo (now Democratic Republic of the Congo), tested ten organophosphorus insecticides to see whether any of them could destroy samples of *Ascaris lumbricoides* that he had cultured in Ringer’s solution. After finding metrifonate to be active, he arranged for the commercial powder to be formulated in tablets at a local pharmacy. Cerf then experimented on himself to establish a safe dose and gave this amount to 15 volunteers whom he carefully monitored for toxic effects. Cerf went on to conduct a trial on 2000 patients, most of whom were infected with *Ascaris* or *Anchylostoma duodenale*. From this, it became clear that metrifonate was a highly effective anthelmintic agent. Trials in Egypt subsequently established that metrifonate could also eradicate bilharzia caused by *Schistosoma haematobium*. It became the drug of choice for treating bilharzia until praziquantel was introduced.

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Drugs Discovered through Serendipity in the Laboratory

The previous chapter was concerned with the serendipitous discovery of drug prototypes after observations were made on people or patients exposed to chemical compounds. However, the most celebrated serendipitous discovery of all – the discovery of penicillin – was made in the laboratory. It was, however, by no means the sole discovery of that type. Others are now discussed.

ACETANILIDE

The department of internal medicine at the University of Strassburg in the 1880s was noted for its investigations into intestinal worms. Adolf Kussmaul, the director, asked two young assistants, Arnold Cahn and Paul Hepp, to treat patients with naphthalene as it had been used elsewhere as an internal antiseptic. The young doctors were disappointed with the initial results, but Hepp persevered with the naphthalene treatment in a patient suffering from a variety of complaints besides worms. Surprisingly, the fever chart revealed a pronounced antipyretic effect from this treatment. This had not been observed before, but further investigation revealed that Hepp had wrongly been supplied by Kopp’s Pharmacy in Strassburg with acetanilide instead of naphthalene! Cahn and Hepp lost no time in publishing a report on their discovery of a new antipyretic. This appeared in August 1886, and a small factory, Kalle and Company, situated outside Frankfurt set up in competition with Hoechst’s Antipyrin (i.e. phenazone), mischievously calling their product Antifebrin. In 1908, however, the Farbenfabriken Hoechst obtained control of Kalle and Company, which had grown considerably in size largely due to the success of their antipyretic. As acetanilide was cheaper to manufacture than other antipyretics, it remained in use for many years, despite the fact that it inactivated some of the haemoglobin in red blood cells, a medical condition known as methaemoglobinaemia. Sometimes acetanilide was used illicitly as a cheap adulterant of other antipyretics.

Immediately after the publication of the report of the antipyretic activity of acetanilide, Carl Duisberg, chief research chemist at F. Bayer & Company in Elberfeld, decided that its 4-methoxy and 4-ethoxy derivatives should be prepared. He assigned the task to Otto Hinsberg, a lecturer at the University of Freiburg who was working at Elberfeld during the summer vacation. When the task was completed, Hinsberg gave the two new compounds to the Professor of Pharmacology at Freiburg, Alfred Kast. Kast then demonstrated that both

[Structural formulas of acetanilide, acethophenetidin, and paracetamol are shown]
were antipyretics, noting that the ethyl ether was less toxic than acetanilide.\textsuperscript{2} It was promptly put on the market as Phenacetin\textsuperscript{1}, a proprietary name that suffered a similar fate to Aspirin\textsuperscript{1} at the end of the First World War. In countries where this name continued to be recognised as the property of the Bayer Company, the approved name became acetophenetidin. It became a highly successful product, establishing F. Bayer & Company as a leading pharmaceutical manufacturer. Acetophenetidin remained popular for about 90 years until mounting concern about kidney damage in chronic users led to restrictions on its supply.

Many attempts were made to find an antipyretic superior to phenacetin. The eminent clinical pharmacologist Joseph von Mering collaborated with the Bayer Company in a trial of paracetamol in 1893. He found it to be an effective antipyretic and analgesic, but claimed that it had a slight tendency to produce methaemoglobinaemia. This could conceivably have been caused by the contamination of his paracetamol with the 4-aminophenol from which it was synthesised. Such was the reputation of von Mering that nobody challenged his observations on paracetamol until half a century later, when Lester and Greenberg\textsuperscript{3} at Yale and then Flinn and Brodie\textsuperscript{4} at Columbia University, New York, confirmed that paracetamol was formed in humans as a metabolite of phenacetin.

In 1953, paracetamol was marketed by the Sterling–Winthrop Company. It was promoted as preferable to aspirin since it was safer in children and anyone with an ulcer. Time has shown that it is not without its disadvantages, for it is far more difficult to treat paracetamol poisoning than that caused by aspirin.

It is fair to say that if an attempt were to be made today to introduce either paracetamol or aspirin into medicine, they might be denied a license. Nevertheless, when used in moderation they remain a boon to mankind.

**ANTICONVULSANTS**

During the late 1930s there was some interest in a class of sedative-hypnotics known as oxazolidine-2,4-diones. One of these, propazone, proved to be an anticonvulsant, but its potent sedative activity ruled out any clinical application. At the Abbott Laboratories in Chicago, tests confirmed that none of the known oxazolidine-2,4-diones had any analgesic activity. However, Marvin Spielman found that when the lipophilicity of these compounds was increased by substituting a methyl group on the nitrogen atom, analgesics comparable with aspirin were obtained.\textsuperscript{5}

In the course of trials to establish its clinical value, the most promising of the new analgesics, troxidone, was combined with a novel antispasmodic drug, amolanone. Toxicological studies in mice revealed that large doses of the antispasmodic that would normally induce convulsions did not do so when troxidone was concurrently administered. The obvious conclusion was drawn, namely that troxidone was an anticonvulsant. This discovery was made in 1943, and after extensive animal investigations the new anticonvulsant was administered the following year to children at the Cook County Hospital in Chicago. The trial established that, unlike phenytoin or phenobarbital, troxidone was capable of controlling petit mal absence seizures. It was the first drug ever to do this, but it caused many side effects. Nevertheless, troxidone was a turning point in the development of anticonvulsant drugs because it showed that these could be selective in their spectrum of activity. Henceforth, a battery of animal tests were set up for screening potential new anticonvulsants.
ALKYLATING DRUGS

Early in 1942, Yale University entered into a contract with the US Office of Scientific Research and Development, whereby Louis Goodman and Alfred Gilman agreed to investigate the pharmacological action of the recently developed nitrogen mustard chemical warfare agents. They were surprised to discover that the damage done to an animal after a nitrogen mustard was absorbed through its skin into the circulation was of greater consequence than the blistering action on initial skin contact. The toxicity was most extensive in rapidly dividing cells, notably the blood-forming elements in the bone marrow, lymphoid tissue and the epithelial linings of the gastrointestinal tract. The consistency of this phenomenon persuaded Goodman and Gilman to invite their colleague Thomas Dougherty to examine the influence of nitrogen mustards on transplanted lymphoid tumours in mice.

After making preliminary checks to establish a non-lethal dose range in normal mice, Dougherty administered a nitrogen mustard to a single mouse bearing a transplanted lymphoma that was expected to kill the animal within three weeks of transplantation. After only two injections had been administered, the tumour began to soften and regress, subsequently becoming unpalpable. On cessation of treatment, there was no sign of its return until a month had passed, whereupon it gradually reappeared. A second course of injections afforded a shorter respite than before; the lymphoma ultimately killed the mouse 84 days after transplantation. Such an unprecedented prolongation of life was never matched in subsequent studies on a large group of mice bearing a variety of transplanted tumours, although good remissions were frequently obtained. This was correctly seen by Goodman and Gilman to indicate a varying susceptibility of different tumours to specific chemotherapeutic agents, a view that did not accord with the perceived wisdom of the time. There was, however, no doubting the therapeutic implication of their results on animals, and in August 1942 treatment of a patient in New Haven Hospital began, under the supervision of an assistant professor of surgery at Yale. A 48 year old silversmith was dying from a radiation-resistant lymphoma that had spread over his chest and face, preventing chewing or swallowing, and causing considerable pain and distress. A nitrogen mustard, code-named HN3 (viz. 2,2',2''-trichlorotriethylamine), was administered at a dose level corresponding to that previously used for mice. Belatedly, this was found to be somewhat high, resulting in severe bone marrow damage. Nevertheless, the patient survived a full ten-day course of injections. Despite his apparently hopeless condition at the onset of therapy, he responded as dramatically as the first mouse had. An improvement was detected within two days, when the tumour masses began to shrink. On the fourth day the patient could once again swallow, while after two weeks there were no signs of any tumour masses. Bone marrow cells began to regenerate over a period of weeks, but so too did tumour masses. A brief second course of injections was of some value, but a third course failed to prevent the lethal progress of the disease.6

A further six terminally ill patients with a variety of neoplastic diseases were treated at New Haven before the nitrogen mustard group at Yale was disbanded in July 1943. Earlier that year, Charles Spurr, Leon Jacobson, Taylor Smith and Guzman Barron of the Department of Medicine at the University of Chicago began a full-scale clinical trial of another nitrogen mustard, then known as HN2 but later given the approved name of 'mustine', which has since been changed to chloromethine. They examined its effects on 59 patients with various blood dyscrasias and obtained spectacular remissions in patients with Hodgkin’s disease, among whom were several who had ceased to respond to X-ray therapy. When Cornelius Rhoads, chief of the the Army Chemical Warfare Service based at Wedgewood Arsenal in Maryland, was informed of the results of this trial in August 1943, he arranged for further secret clinical trials in various American hospitals. Rhoads was on wartime leave of absence from his post of Director of the Memorial Hospital in New York, a leading cancer treatment centre. When the results of all the secret trials had been collated, a contract was awarded for David Karnovsky
and his colleagues to organise a major clinical trial at the Memorial Hospital in order to establish the relative merits of HN2 and HN3 in patients with leukaemia, Hodgkin’s disease and brain tumours.

Because of the strict wartime secrecy surrounding all work on nitrogen mustards, no information about any of the trials was released until 1946. It was then revealed that HN2 and HN3 had produced useful results in patients with Hodgkin’s disease, lymphomas or chronic leukaemias, although there was doubt concerning whether they had any superiority over X-rays properly applied to solid tumours. Poor results had been obtained in patients with acute leukaemia, although partial remissions occurred in some cases. Those with other neoplastic diseases failed to respond. On balance, mustine seemed a better drug than HN3.

During the war, information on nitrogen mustards had been freely exchanged between British and American investigators holding government contracts. Chemists George Hartley, Herbert Powell and Henry Rydon at Oxford University had obtained experimental proof that the chloroethyl side chain in these compounds cyclised to form highly reactive aziridine ions that could rapidly alkylate vital tissue components. It is now known that inhibition of cell division occurs because DNA is alkylated by nitrogen mustards on guanine at N-7 and on adenine at N-3, with cross-linking from guanine to guanine or guanine to adenine then occurring. Consequently, the generic term for anticancer drugs that act in this manner is ‘alkylating drugs’.

It was assumed that only compounds capable of forming aziridine ions could be effective alkylating drugs. That this was not necessarily so first became apparent in 1948 when Alexander Haddow, George Kon and Walter Ross at the Chester Beatty Institute (the research division of the Royal Cancer Hospital in London) discovered that aromatic nitrogen mustards were effective cytotoxic agents, despite being unable to form aziridine ions. The following year, Reginald Goldacre, Anthony Loveless and Ross published a paper suggesting that the cytotoxic action of both aliphatic and aromatic nitrogen mustards might be due to their ability to cross-link cellular components such as the nucleic acids, and that it was not essential for aziridine ions to be formed for this to occur. All that was necessary was the presence of two chemically reactive functional groups. The publication of this paper stimulated the development of several useful drugs.

Walter Ross decided to investigate the action of diepoxides that might conceivably act as cross-linking agents in a manner akin to that of the nitrogen mustards. Before he had an opportunity to put this to the test, John Speakman at the Department of Textile Industries at the University of Leeds suggested to Haddow that the biological properties of cross-linking agents used in textile technology should be examined, especially those of the diepoxides. Speakman had become interested in the work at the Chester Beatty Institute after examining the ability of aromatic nitrogen mustards to cross-link keratin fibres. A series of diepoxides subsequently prepared by James Everett and Kon turned out to act almost identically to the nitrogen mustards. However, Haddow’s group were not alone in discovering this. Francis Rose and James Hendry at the ICI research laboratories in Manchester had examined polymethylolamides used as cross-linking agents in paper and textile technology, fields in which their company had considerable expertise. The only compound with worthwhile cytotoxic activity turned out to be the product of condensing formaldehyde with melamine. The ICI researchers then tested epoxides and ethylene imines, also used to cross-link textile fibres. Of the former class, one of the most active compounds was diepoxybutane, which
consisted of a mixture of isomers. This was one of the compounds that Ross at the Chester Beatty had examined, but it had not been considered suitable for clinical application. It was not until 1960 that Walpole found a diepoxide that was suitable, namely etoglucid.\textsuperscript{13} It was subsequently marketed and used for a number of years in the treatment of bladder cancer.

![Etoglucid](image)

The most potent alkylating agent investigated by ICI was tretamine, a compound used in the textile industry to cross-link cellulose fibres. It had three aziridine rings attached to a triazine ring. On investigation, it turned out to be suitable for treating lymphatic and myeloid leukaemias, as well as Hodgkin’s disease.\textsuperscript{14} As it lacked the extremely high chemical reactivity of the nitrogen mustards, tretamine became the first alkylating drug that could be given by mouth.

![Tretamine](image)

![Thiotepa](image)

In 1950, after the ICI group had prepared, but not yet published, their first paper reporting their work on tretamine,\textsuperscript{15} Joseph Burchenal and Chester Stock of the Sloane–Kettering Institute for Cancer Research in New York, in conjunction with Moses Crossley, the chief chemist at the Bound Brook laboratories of the American Cyanamid Company, published a prior report describing the action of tretamine against experimental tumours.\textsuperscript{16} The following year, the Sloane–Kettering researchers introduced thiotepa, an alkylating drug synthesised by Crossley and his colleagues.\textsuperscript{17} It is still used in the treatment of malignant effusions, as well as ovarian and bladder cancer.

Three frequently prescribed alkylating drugs were synthesised and evaluated at the Chester Beatty laboratories and marketed by Burroughs Wellcome. The first was busulphan, prepared in 1950 by Geoffrey Timmis, a former employee of Burroughs Wellcome.\textsuperscript{18} It was the most potent of a series of sulfonic acid esters in which alkylsulfonyl functions could undergo nucleophilic displacement to act as alkylating agents. Clinical studies completed in 1953 confirmed that, when given by mouth, busulphan had a selective action on the blood-forming cells of the bone marrow and was effective in the treatment of chronic myeloid leukaemia.

![Busulphan](image)

![Chlorambucil](image)

![Melphalan](image)

![Estramustine](image)
The next important cross-linking agent developed at the Chester Beatty laboratories was chlorambucil. Recognising that the clinical value of aromatic nitrogen mustards previously prepared was circumscribed by lack of specificity of action, Ross exploited a concept enunciated half a century earlier by Paul Ehrlich, who had pointed out that introduction of acidic or basic groups into dyes and other molecules greatly influenced their ability to penetrate tissues. By adding acidic side chains to aromatic mustards he hoped to obtain a less toxic drug. This objective was achieved when chlorambucil was shown, in 1952, to be less toxic to the bone marrow than was chlormethine. Since then it has been widely used in the treatment of chronic lymphocytic leukaemia and ovarian cancer.

The third alkylating drug developed at the Chester Beatty laboratories was melphalan, the synthesis of which was reported by Franz Bergel and John Stock in 1954. This represented a further refinement of the design approach that led to the synthesis of chlorambucil. This time, the extra moiety incorporated in an attempt to improve tissue selectivity was alanine, a natural amino acid. It was chosen to render the polar drug similar to phenylalanine in the hope that it would enter target cells through the active transport pathway for phenylalanine. The approach succeeded and melphalan has proved to be a valuable drug in the treatment of multiple myeloma.

The success of the approach taken at the Chester Beatty laboratories encouraged researchers throughout the world to synthesise a vast range of nitrogen mustard analogues incorporating different biological carriers. Despite the expenditure of much effort, little was gained from this. The only drug of note to be developed was estramustine, which contained a nitrogen mustard function attached to oestradiol. It was developed by Niculescu-Duvaz, Cambani and Tarnauceanu at the Oncological Institute in Bucharest in 1966, and is used in the treatment of prostatic cancer. The elaboration of another nitrogen mustard for treatment of prostatic cancer was based on a different, but flawed, approach. This was cyclophosphamide, developed in 1956 by Herbert Arnold, Friedrich Bourseaux and Norbert Brock of Asta-Werke AG in Brackwede, Germany. The idea behind its synthesis was the same as that which had previously led Arnold to obtain a patent on the use of fosfestrol, the diphosphate ester of stilboestrol. He believed that this was devoid of hormonal activity until it decomposed in the presence of acid phosphatase, an enzyme present in prostatic tumours. Since large amounts of this enzyme were released into the circulation in patients with prostatic cancer, it is hardly surprising that there is little evidence to support the contention that fosfestrol is superior to stilboestrol. In cyclophosphamide, a nitrogen mustard function was combined with a phosphoramidate residue in the hope that there would be no significant alkylating activity until enzymic action decomposed the drug. Paradoxically, cyclophosphamide turned out to have good activity against a wide variety of malignancies and chronic lymphocytic leukaemia, but not against prostatic tumours. Ten years after its introduction, Brock found that this was because rather than being decomposed by acid phosphatase, as originally hypothesised, the drug was metabolised by liver enzymes and thereby converted to an active species.

Asta-Werke introduced an isomer of cyclophosphamide known as ‘ifosfamide’ in 1967. It has similar therapeutic activity.
VALPROIC ACID

Pierre Eymard, a research student at the University of Lyon, synthesised a series of derivatives of khellin as part of his doctoral studies. After completing his thesis he arranged to have his new compounds evaluated, but when he tried to prepare a solution of the first compound to be tested he could not get it to dissolve. He then sought advice from Hélène Meunier of the Laboratoire Berthier in Grenoble. She suggested that valproic acid might be a suitable solvent as she had used it in the past to dissolve bismuth compounds for clinical evaluation.

The valproic acid did dissolve Eymard’s compound and subsequent tests showed the khellin derivative to have anticonvulsant activity. Shortly after this, Meunier used valproic acid to dissolve a coumarin compound unrelated to Eymard’s compound. When it also proved to have anticonvulsant properties, she suspected this was not mere coincidence. She immediately tested the valproic acid and discovered that it was an anticonvulsant. After detailed investigations, valproic acid was subjected to extensive clinical evaluation before its sodium salt was marketed in 1967 for the control of epileptic seizures.25

REFERENCES


Experimental pharmacology is an area of science that could only develop once pure chemicals of consistent quality had been isolated. Pharmacologists used live animals and isolated tissues to evaluate the alkaloids and glycosides isolated early in the nineteenth century and the synthetic drugs that followed them. In the field of chemotherapy, Ehrlich infected animals in order to screen for antitrypanosomal drugs, an approach that was also to prove successful when applied to the development of the synthetic antimalarials and sulfonamides. However, by the end of the twentieth century, many companies were deserting the traditional approaches involved in screening compounds. Instead, the search for novel drugs now embraces all that modern science can offer, as seen with the dual application of combinatorial chemistry and high-throughput screening. This harnesses the power of the computer to organise massive programmes in which hundreds of thousands of chemicals prepared by combinatorial chemistry, a form of robotic unitary construction involving the attachment of variant chemicals on to molecular templates, are routinely screened for activity in multiple arrays of protein targets identified by DNA technology and the like. Hundreds of thousands of different molecules can now be prepared and screened in a matter of weeks, but success in the field of drug research is not measured by the degree of its sophistication. The first decade of this new methodology has failed to live up to its promise and fewer novel drugs have been discovered during this period than in the other decades since the 1950s. In fairness to those who are labouring to overcome this disappointment, it has to be said that the prime reason for it arises from a decline in the number of novel drug prototypes from botanical and biochemical sources. Drug research in the twentieth century was driven by the development of analogues of alkaloids, hormones and similar natural substances, only a few of which have been isolated during the last 50 years. It is to be hoped that the conclusion of the human genome project will remedy this situation in years to come if novel enzyme targets for drug design evolve from it. The prospects for gene therapy, on the other hand, have been seriously damaged by the inability of pharmaceutical scientists to develop safe methods for its delivery.

In the interim period before new biochemical target molecules are discovered, there remains a serious threat to the development of new drugs that could have unforeseeable consequences if it is not addressed. Reflection on what has happened over the last two centuries reveals that it is relatively easy to discover a new drug, but exceedingly difficult to discover one that is safe enough to be administered as a medicine to heal the sick. The previous pages have described many successes of modern drug research. It would have required several more volumes if consideration had also been given to the far greater number of projects that failed.

The reality of the situation is that when any foreign substance is introduced into the body there will always be a risk of some unanticipated reaction occurring that existing safety tests cannot detect. The demands made upon the pharmaceutical industry to ensure that its products are safe are quite understandable, but the level of sophistication of current pre-launch chronic safety testing and post-marketing surveillance of patients now means that every new product faces the risk of being withdrawn shortly before or after its launch. Hundreds of millions of pounds or dollars will have been spent by this time, and much greater
expense may also be incurred to meet claims for compensation if a drug has to be withdrawn after it has been marketed. It is inevitable that all these costs will continue to rise and a time may soon come in which the commercial risks of developing new drugs will be considered too great when measured against the potential financial returns. Unless steps can be taken to resolve this issue, the prospects for drug discovery in the future could be bleak.

The public needs to be better educated about the nature of drug therapy. The message that must be put across is that no matter how carefully and conscientiously a pharmaceutical company designs, selects and evaluates drugs in order to introduce one that fulfils its intended medicinal role by affecting one specific target, the human body is so complex that there will always be the possibility of an unintended target also being hit. Even when the best practice in conducting safety tests on animals, volunteers and those patients involved in early trials has been followed to the point of perfection, the potential for disaster will still persist through no fault of those involved. By agreeing to accept any medication that has been exhaustively tested and correctly administered in the light of existing knowledge, patients should be considered to have accepted this minimal risk. If they are to be compensated for any damage to their health that arises, the settlement should take acceptance of that risk into account. Whether or not the government under whose authority the drug received a license indicating that it had been thoroughly tested should pay compensation could determine the likelihood of future drugs ever reaching the market. A levy on the sale of all drugs, including generics no longer covered by patent, would cover the cost of compensating the few who are damaged by the drugs that help restore the good health of so many.