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.¹ 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.¹ 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

©2005 John Wiley & Sons Ltd
were antipyretics, noting that the ethyl ether was less toxic than acetanilide. It was promptly put on the market as Phenacetin, a proprietary name that suffered a similar fate to Aspirin 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 at Yale and then Flinn and Brodie 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.

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. It was subsequently marketed and used for a number of years in the treatment of bladder cancer.

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. As it lacked the extremely high chemical reactivity of the nitrogen mustards, tretamine became the first alkylating drug that could be given by mouth.

In 1950, after the ICI group had prepared, but not yet published, their first paper reporting their work on tretamine, 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. The following year, the Sloane–Kettering researchers introduced thiotepa, an alkylating drug synthesised by Crossley and his colleagues. 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. 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.
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


