THREE CRUCIAL SCIENTIFIC OBSERVATIONS FROM MISTAKEN HYPOTHESES

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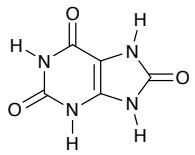
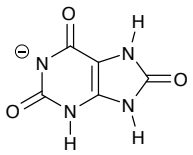
The Treatment of Mania with Lithium

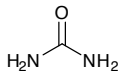
In 1949 John Cade, a senior medical officer of the Victorian Department of Mental Hygiene, Australia, investigated the effects of lithium carbonate and lithium citrate on the mental state of patients suffering from mania or manic depression (1). This illness is sometimes known as bipolar disorder. Lithium was regarded as a controversial medication, which had resulted in the deaths of some patients and could sometimes cause unpleasant side effects. In the 19th century it was used for the treatment of gout and other ailments following the discovery by Garrod that lithium urate, a salt of uric acid, was the most soluble alkali metal urate. The presence of crystalline monosodium urate monohydrate (2) in the human body causes acute gouty arthritis, so the traditional use of lithium as a remedy for this has a theoretical basis. Pieces of cartilage with uric acid deposits were immersed in solutions of sodium, potassium, and lithium carbonate. Lithium carbonate was the fastest solution to dissolve the deposits. Enthusiasm for its use extended into other medications and also led to the widespread use of bottled curative waters, some of which are still marketed today, such as Perrier and Vichy, promoted at one time for their lithium content.



uric acid



(lithium) urate



urea

Cade carried out an experiment to determine whether uric acid was a toxic component of urea in manic patients. He wanted to determine whether this component might be the cause of mania. Unlike urea, uric acid is only poorly soluble in water, so the most soluble urate, lithium urate, was chosen. An aqueous solution of 8% urea saturated with lithium urate was injected intraperitoneally into some guinea pigs. The toxicity was less than expected. It appeared that the lithium might be exerting a protective effect, so further experiments were performed. Lithium urate was replaced with lithium carbonate. An 8% aqueous solution of urea kills five out of ten guinea pigs when injected intraperitoneally in doses of 1.25 mL per ounce of body weight. When 0.5% lithium carbonate in an 8% urea solution was injected in the same dosage, all ten animals survived. This showed that the lithium ion itself had a protective function against the convulsant mode of death caused by toxic doses of urea. This was an interesting and unexpected discovery which led to the next key experiment.

Cade proceeded to determine the effect of lithium salts alone on guinea pigs. When some animals were injected with large doses of 0.5% aqueous lithium carbonate solution, a key observation was made. After a period of about two hours the animals, still conscious, became lethargic and

unresponsive to stimuli for a few hours before returning to normal. Cade commented (1):

> It may seem a long distance from lethargy in guinea pigs to the excitement of psychotics, but as these investigations had commenced in an attempt to demonstrate some possibly excreted toxin in the urine of manic patients, the association of ideas is explicable.

Following this observation Cade wanted to try to use lithium salts for the treatment of mania. He also concluded that certain waters of wells in the British Isles, which were considered to have special virtue in the treatment of mental illness, had a real efficacy proportional to the lithium content of the waters. Ten manic patients were treated with either lithium carbonate or lithium citrate with remarkable results. The case study for one of them is reported below.

CASE II- E. A male, aged forty-six years, had been in a chronic manic state for five years. He commenced taking lithium citrate, 20 grains three times a day, on May 5, 1948. In a fortnight he had settled down, was transferred to the convalescent ward in another week, and a month later, having continued well, was permitted to go on indefinite trial leave whilst taking lithium citrate 10 grains three times a day. This was reduced in one month to 10 grains twice a day, and two months later to 10 grains once a day. Seen on February 13, 1949, he remained well and had been in full employment for three months.

All ten case studies are reported in Cade's original publication (1). Every patient showed a tremendous improvement. Lithium was introduced into medicine through a mistaken hypothesis. Today it is known as a "mood stabilizer" for people with mania. It serves to stabilize mood cycles by dampening high periods and easing low or depressive mood. Fortunately, some compounds that were developed for epilepsy like carbamazepine, trade mark Tegetrol, also function as mood stabilizers and can be taken alongside lithium for even better mood control (3). Lithium has enabled many individuals who suffer from manic depression to live relatively normal lives.

Cisplatin (II)

An in situ Drug from a Platinum Electrode

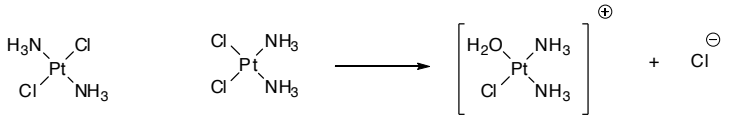
In 1965 the scientists Barnett Rosenberg, Loretta Van Camp, and Thomas Krigas of the Biophysics Department at Michigan State University, USA, investigated the effect of an electric current upon the growth of a suspension of bacteria (4). A special culture chamber was designed which contained platinum mesh electrodes. The chamber was charged with a nutrient medium and inoculated with a species of *Escherichia Coli* and the bacterial population allowed to reach a steady state. The electric field was turned on at 1,000 cycles/sec for two hours. Platinum was chosen as the electrode material because of its chemical inertness, and the field was chosen to eliminate electrolysis effects and electrode polarization. Although these two precautions were taken, the scientists wrote (4):

> As we will show, both are mistaken ideas which led, via serendipity, to the effects described in this communication.

Microscopic examination of the effluent from the chamber showed that the *E. Coli* had ceased dividing and had begun to elongate. After just a few hours of electrolysis all the bacteria were in the form of long filaments that continued to increase in length rapidly with time. Even if the electric current was switched off the bacteria *continued to increase in length*. Oxygen was required to produce the effect. If nitrogen or helium was bubbled through the cell, the electric current had no effect upon the bacterial culture. Unexpectedly, a frequency of 500 c/s was the most effective in causing filamentous growth compared to the highest frequency used of 6,000 c/s. The lower frequency may allow more time for an active species to diffuse from the electrode surface so the concentration increases.

The filamentous growth observed by the scientists involved an inhibition of cell division but not of cell growth. In other words, the cells keep on stretching as they grow longer without dividing. Some agents known to cause filamentous growth were eliminated as possible causes here. These were ultraviolet light, temperature, pH, and magnesium ion. The investigators considered that a new chemical species might be generated in the electrolysis chamber, which was the causative agent. To test this hypothesis the nutrient medium was pumped into an electrolysis chamber and electrolyzed, after which it was pumped into a bacterial chamber with no electrodes. The electric current was only passed through the electrolysis chamber. If a new chemical species were formed in the nutrient medium in the electrolysis chamber that had a sufficiently long life time, it would still cause filamentous growth when added to the bacterial chamber. This proved to be the case. It caused elongation in the bacterial chamber, provided oxygen was present in the electrolysis chamber. If helium was bubbled through the electrolysis chamber, no elongation of cells occurred when the nutrient was transferred into the bacterial chamber.

The investigators showed by using a potassium iodide-starch test that an oxidant was being generated in the electrolysis reaction. Ordinary medium gave no reaction whereas the electrolyzed medium gave a positive test by turning from yellow to orange to blue after about five minutes. This oxidant might be the unknown *in situ*- formed reagent. A series of sensitive qualitative tests were used to detect possible oxidizing ions, all of which proved negative. Previously the investigators had passed an electric current through the nutrient medium. Now they took the known individual components of the medium, electrolyzed them separately, and tested for an oxidant. Results were negative with phosphate, sulfate, phosphate and glucose, phosphate, sulfate and glucose, sodium sulfate, and sodium carbonate. Positive results were obtained with ammonium and other chlorides. These solutions with chloride anions showed the characteristic yellow to orange to blue color changes with the starch/potassium iodide test. It was known that platinum electrodes can be attacked by an acidified chloride solution during electrolysis to form a compound of the formulae $[PtCl_6]$. A soluble platinum salt might therefore be the active agent. A solution of $(NH_4)_2PtCl_6$ tested positive with the potassium iodide/starch test, thus duplicating the series of color changes seen with the electrolyzed medium. Most importantly, inoculation of the bacterial culture chamber with a solution of $(NH_4)_2PtCl_6$ caused filaments to appear. The chemical needs only to be about 10 ppm to exert an effect. In the electrolysis experiments oxygen is vital and must somehow assist in the oxidation of the platinum electrode to generate $(NH_4)_2PtCl_6$.



Transplatin (inactive) Cisplatin (active) Active anti-tumor species

Cisplatin and the Active Anti-tumor Species

From this breakthrough discovery a series of platinum compounds were tested for anti-tumor activity (5). Clinical trials began in the early 1970s with *cis*-Pt(II) $(NH_3)_2Cl_2$, with the outcome that cisplatin has become established as a valuable agent in the treatment of malignant teratoma or cancer of the testes. The compound $(NH_4)_2PtCl_6$, deduced to be present in the electrolysis nutrient, is in a higher oxidation state (Pt IV) and has a different structure from cisplatin; but since it is a mild oxidant it may get reduced *in situ* to generate square planar cisplatin.

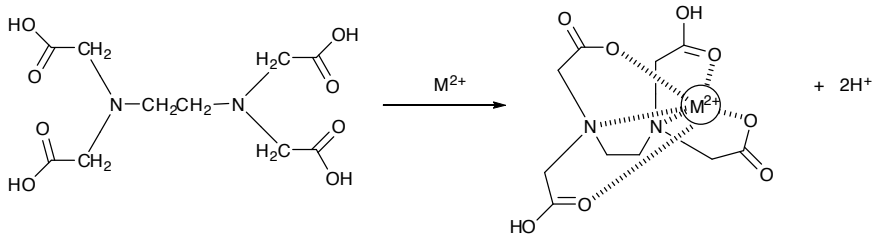
Since the discovery that cisplatin can interfere with cell division, investigators have tried to unravel the mechanism by which it works. As a neutral compound, it can readily cross cell membranes into cells. The interior of a cell membrane bilayer is nonpolar because of the lipid hydrocarbon chains, which can absorb neutral or nonpolar drugs like cisplatin. However, the chloride ligands are quite labile. The chloride ion concentration within cells is lower (4 mM) than in blood (104 mM). This favors hydrolysis and replacement of one of the chloride anions with water to give $[Pt(NH_3)_2(H_2O)Cl]^+$. This is an active species of the drug, which is a stronger coordinating agent because it is positively charged. Current opinion is that the platinum metal binds to some of the bases of a DNA strand and may bridge between them. In doing so it prevents the DNA from separating into strands, which is required for cell division to occur. It may work by changing the shape of the double helix and thus preventing a vital enzyme from recognizing the base-pair sequence.

In contrast to the flat *cis* isomer, the *trans* isomer exhibits no anti-tumor activity.

A New Drug for Treating Cancer

Heterocycles from EDTA

In 1969 the scientists Andrew Creighton, Kurt Hellmann, and Susan Whitecross, working at the Imperial Cancer Research Fund's department of chemistry and cancer chemotherapy, discovered anti-tumor activity in a series of compounds called *bis*-diketopiperazines (6). The initial hypothesis for discovering a new drug was to use a chelating agent that would bind to, and hence inactivate, trace metals that are vital to many enzymes inside cells. The cells would therefore die. The diagram shows how EDTA might bind to divalent metal ions M(II) by using its carboxylate side chains like claws to hold onto a metal ion, as depicted in a simplified X-ray crystal structure drawing of a metal complex of EDTA (7). Two carboxylate groups are ionized which balances the charge of the metal-ion in the center. The two nitrogen atoms which have lone pairs of electrons also coordinate making the complex even stronger. In total seven ligands bind to the metal ion: two carboxylates, two carboxylic acids, two tertiary nitrogen atoms, and a water.



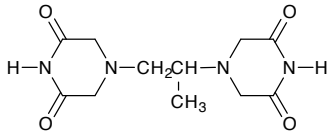
EDTA Possible EDTA metal complex

EDTA Binding a Metal-ion

EDTA, one of the strongest chelating agents for divalent cations, has no significant anti-tumor activity. This was readily rationalized because it is a charged polar molecule that would not easily cross through cell membranes. If a drug is to be absorbed and pass through a cell membrane into a cell, it should be nonpolar and quite "greasy." The investigators reasoned that if they prepared less polar derivatives of EDTA, these might penetrate cells more readily and break down into EDTA once inside the cell. These derivatives of EDTA would be *latent* precursors that are masking the desired molecule. The first two compounds synthesized and tested—the methyl and ethyl derivatives of EDTA—were inactive. The team then studied the reaction between EDTA and formamide in an attempt to prepare the tetramide. However, an unexpected product was formed: a diimide that showed promising screening results (8). This procedure is in fact a modification of one by J. R. Geigy (9), in which an extensive series of poly-N-diacetic acid imides are described as levelling agents, intermediates, textile auxiliaries, and curing agents. Both formamide, acetamide and urea can be used. Hence this very unusual reaction to convert iminodiacetic acids into diketopiperazines was already patented but for a different application.

Because of this discovery some further derivatives were made (10). A variety of acyclic analogs, in which the six-membered rings had been opened up, were inactive. This showed that the rings were important for the biological activity inside the cells and that they were not just allowing the compound to pass through the cell membranes. The incorporation of a methyl group—but not an ethyl group—at the central ethylene chain retained activity. These studies showed that a highly specific structure was required for anti-tumor activity. After publication of these results in 1969 the compound ICRF 159 was put on clinical trial. This led to its introduction for the treatment of acute myeloid leukemia and non-Hodgkin lymphomas (all forms of cancer) under the trade name of razoxane or razoxin.

This drug has no metal-ion chelating properties but was synthesized as part of a program to find a metal-ion chelating anti-tumor agent. The four arms of EDTA have been bound into six-membered heretocyclic rings with imide nitrogen atoms. These are less acidic than carboxylic acids, and geometry prevents them from simultaneously wrapping around a metal-ion like the carboxylic acids could. By an amusing twist of fate it was published in the journal *Nature*, along with the first metal-based compound to have anti-tumor activity, cisplatin.



ICRF 159 Razoxane

Summary

Three examples of serendipitous discovery have been uncovered by the author after an extensive search of the literature over a number of years. They were selected from about 20 interesting examples, although a search engine analysis of the wider literature reveals hundreds of examples of publications with the word serendipity in the title or text. The common occurrence of serendipity in medicine, which is still underpinned by chemistry, is highlighted. Lithium and cisplatin (II) are inorganic compounds, whereas a *bis*-diketopiperazine is an organic compound that was prepared by chemical synthesis. There are numerous foundations and charities that fund medical research, probably because the public appreciate their role in health care and will provide financial support. But the public may not so easily grasp the role of chemistry in a medical discovery and hence may not appreciate its importance. Serendipity can help profile both chemical and medical research to a wider audience, and these examples illustrate that it can save lives and enhance the quality of life through anti-cancer therapy and the treatment of manic patients. The papers may have been based on mistaken hypotheses because the research plans were exploratory with an element of risk; but that thinking led to innovative discoveries. This

short synopsis highlights the need to fund basic blue sky research and to allow the development and exploitation of unexpected observations.

Origins of the Word Serendipity (11)

Horace Walpole (1717-97) fourth Earl of Oxford, son of Prime Minister Robert Walpole, connoisseur, antiquarian, and author of the famous gothic novel *The Castle of Otranto* and the 48-volume *Horace Walpole's Correspondence*, is credited with deriving the word serendipity from two letters that he wrote. The first (12), dated and addressed January 28, 1754, Arlington, [Piccadilly, London] was written to Horace Mann, an envoy in the service of King George II stationed in Florence. It was to acknowledge the safe arrival of a portrait of Bianco Capello, a 16th-century beauty and Duchess of Tuscany. His search for both a Capello and Medici coat of arms for the frame of the painting triggered his thoughts on serendipity. He unexpectedly found a Capello coat of arms in a Venetian book with a fleur-de-lis attached to a blue ball. He recognized the fleur-de-lis as a Medici emblem and was persuaded that it was given to the Capello family by the Grand Duke in recognition of the marriage (12):

Horace Walpole, painting by John Giles Exkhardt, 1754

> This discovery indeed is almost of that kind which I call serendipity, a very expressive word…

From here he described an example of serendipity based on a fairy tale called *The Three Princes of Serendip*. The stories were published in Venice in 1557 by a printer Michele Tramezzino, who some believe was also the compiler of these ancient tales. Serendip is an old Persian or Arabic name for Ceylon. The episode with a camel—although Walpole confused it for a mule in his correspondence—inspired Walpole to derive the word serendipity (12):

> I once read a silly fairy tale called *The Three Princes of Serendip*: as their highnesses travelled, they were always making discoveries, by accident and sagacity, of things which they were not in quest of.

Walpole's key definitions for serendipity were "… discovery by accident and sagacity of things you are not in quest of…;" from a second line of thought "…*no* discovery of a thing you *are* looking for comes under this description (12)." An instance of accidental sagacity "…was of my Lord Shaftsbury, who, happening to dine at Lord Chancellor Clarendon's, found out the marriage of the Duke of York and Mrs Hyde, by the respect with which her mother treated her at table."

The second letter, dated and addressed September 10, 1789, Strawberry Hill (14), [Twickenham, London] was written to a social reformer and religious writer Hannah More. He states (13):

> Nor is there any harm in starting new game to invention; many excellent discoveries have been made by men who were *a la chasse* of something very different.

His interest in the scientific method was also expressed here (13):

> I am not quite sure that the art of making gold and of living for ever have been yet found out: yet to how many noble discoveries has the pursuit of those nostrums given birth! Poor chymistry, had she not had such glorious objects in view!

The Oxford English Dictionary definition of serendipity is "The faculty of making happy and unexpected discoveries by accident." Some scholars believe this is different from the original derivation, but the OED has an explanation. Definitions of words can be "how words are or have been used, not how they ought to be used."

Walpole's Gothic mansion is currently under restoration by the Strawberry Hill Trust. Many of the contents, including the original letters, were sold to the Lewis Walpole Library, Farmington, CT/ USA. The Bianca Capello painting was sold from Strawberry Hill in 1842, and its whereabouts is currently unknown.

REFERENCES AND NOTES

1. J. F. J. Cade, "Lithium Salts in the Treatment of Psychotic Excitement," *Med. J. Aust.*, **1949**, *36*, 349-352.
2. N. S. Mandel and G. S. Mandel, "Monosodium Urate Monohydrate, the Gout Culprit," *J. Am. Chem. Soc.*, **1976**, *98*, 2319-2323.
3. K. Inoue, S. Arima, K. Tanaka, Y. Fukui, and N. Kato, "A Lithium and Carbamazepine Combination in the Treatment of Bipolar Disorder—a Preliminary Report, *Folia*

Psychiatrica et Neurologica Japonica, **1981**, *35*, 465-475. In this original paper five case studies were described.
4. B. Rosenberg, L. Van Camp, and T. Krigas, "Inhibition of Cell Division in *Escherichia Coli* by Electrolysis Products from a Platinum Eectrode," *Nature*, **1965**, *205*, 698-699.
5. B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, "Platinum Compounds : A New Class of Potent Antitumour Agents," *Nature*, **1969**, *222*, 385-386.
6. A. M. Creighton, K. Hellmann, and S. Whitecross, "Antitumour Activity in a Series of *bis*-Diketopiperazines," *Nature*, **1969**, *222*, 384-385.
7. A. I. Pozhidaev, N. N. Neronova, T. N. Polynova, M. A. Porai-Koshits, and V. A. Logvinenko, "X-ray Diffraction Investigation of Magnesium Zinc Ethylenediaminetetraacetate Hexahydrate," *Zh. Strukt. Khim.* (Russian) *J. Struct. Chem.*, **1972**, *13*, 690.
8. A. M. Creighton, "Pharmaceutical Compositions," US 4275063, GB 1234935.
9. J. R. Geigy A. G. (a corporate body organized according to the laws of Switzerland); UK patent specification 978,724.
10. S. Sugasawa and H. Shigehara, "Reaction of Formamide. II. Amination of Carboxylic Acids of Formamide," *J. Pharm. Soc. Jpn.*, **1942**, *62*, 532-534; *Chem. Abstr.*, **1951**, *45*, 2861.
11. R. Boyle, http://www.zftrans.com/bbs/simple/index.php?t17617.html and http://livingheritage.org/three_princes.htm.
12. *The Letters of Horace Walpole*, Volume II, pdf books, letter 90 http://pdfbooks.co.za/index.php?option=com_content&task=view&id=7327&Itemid=1
13. *The Letters of Horace Walpole*, Volume IV, Project Gutenberg, letter 346 http://www.gutenberg.org/etext/4919.

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HISTORY OF SCIENCE SOCIETY: http://www.hssonline.org

HISTORY OF CHEMISTRY EuCheMs: http://www.euchems.org

David Katz's "History on Power Points": http://www.chymistry.com