dimensional structure viewer called Cn3d into the Network Entrez client software. This software can be used with MIME-types, to launch from a WWW-browser like RASMOL or MAGE, but it will read ASN.1 data files instead of PDB- or Kinemage-format files. It will also function as a direct Internet client, linking directly to NCBI servers. We will announce the availability of this viewer on the NCBI WWW site (Box 1) and describe its features at a later date.

Acknowledgements

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The maddening business of King George III and porphyria

Alan Bennett's highly successful play and film *The Madness of King George* has re-awakened the debate on whether the sovereign suffered with the haem metabolic disorder porphyria. The original retrospective diagnosis was formulated by Ida Macalpine and Richard Hunter 30 years ago[1]. This article briefly reviews how the diagnosis was reached, our improved understanding of several aspects of porphyria, and the evidence that the disease might have influenced world history beyond that imagined by Macalpine and Hunter.

In 1964, the mother and son combination of Macalpine and Hunter set about the daunting task of writing an account to cover the emergence of psychiatry. As eminent psychiatrists with an established reputation, they made an authoritative team. The fruits of their endeavours were published as a book entitled *Three Hundred Years of Psychiatry, 1538–1860* (Ref. 2). The research had, of course, led to the examination of several prominent figures who had either delved into the budding world of psychiatry, as with James I and his book on witchcraft[2], or had been the study of this evolving subject[3]. What was wrong with George III? Before 1966 it was thought that the King (1738–1820; ascended the throne 1760) suffered from manic depressive psychosis, in which his bouts of madness were brought about by a combination of sexual frustration, self blame and indecision[3–5]. However, the examination undertaken by Macalpine and Hunter failed to classify the King in such a manner, as his conduct did not conform to any known psychiatric disorder. The reality was that George III only suffered from four bouts of mental derangement: October 1788–February 1789; February–March 1801; January–March 1804; and October 1810–January 1820. The total length of his mental incapacity amounted to about six months, up until the time the King entered his eighth decade[1,6]. His final bout coincided with the onset of senility and he was eventually replaced by the Prince of Wales under the regency act of 1811.

On close examination of the material that they had uncovered, Macalpine and Hunter realised that the King's mental problems were ushered in with accompanying physical symptoms, which included acute abdominal pain (Fig. 1), constipation, tachycardia and polyneuritis[2,6]. They quickly recognized that these symptoms, in addition to neurological problems could be attributed to a rare metabolic disorder called porphyria.

Porphyria and tetrapyrroles

Porphyria (derived from *porphuros*, Greek for purple) is a disease caused by defects in the body's ability to make haem[7]. As the prosthetic group of haemoglobin, cytochromes, catalases and peroxidases, haem is an integral component of mammalian systems. It is a modified tetrapyrrole and is related structurally to other biological prosthetic groups such as chlorophyll, cobalamins, factor F430 and sirohaem[8].

The haem biosynthetic pathway is shown in Fig. 2, where 5-aminolaevulinic acid (ALA) is the first committed precursor. Eight molecules of this starting compound are manipulated and transformed into one molecule of haem by the concerted action of seven enzymes[10]. Mutations or deletions in any of these enzymes give rise to porphyria[7] (Fig. 2b). The diseases are generally, though not exclusively, inherited in an autosomal dominant fashion (and thus, heterozygotes have a 50% reduction in the activity of the particular enzyme). Phenotypically, porphyrias are manifested as three types: (1) the so-called acute porphyrias, in which there are severe attacks of abdominal pain, constipation, vomiting, paralysis and psychiatric disorders; (2) the cutaneous porphyrias, in which there is a high degree

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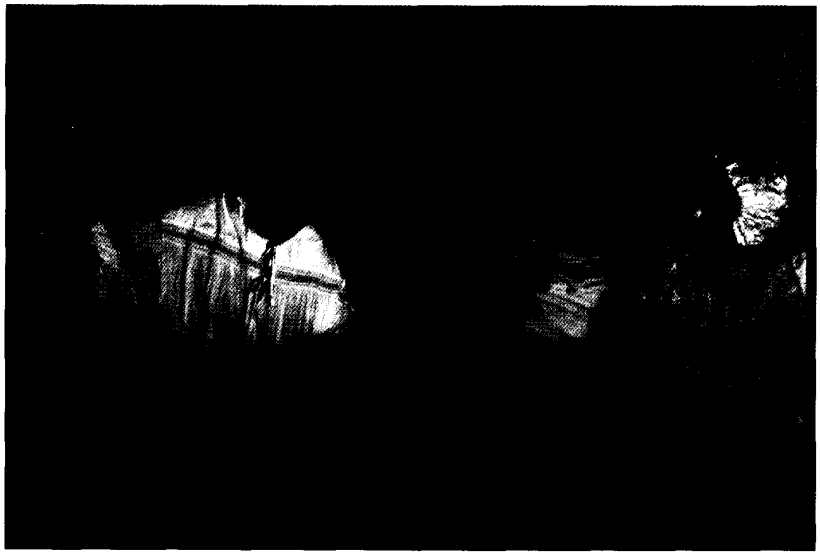

Figure 1

Upon retiring to bed, the King was seen to be in severe discomfort with abdominal pains. Was it something in the pears, which the King had eaten for supper that evening, giving him chronic colic, or were the severe stomach cramps the first sign of an acute attack of porphyria? As the King started to lose control of his senses, the Royal physicians Dr Richard Warren and Sir George Baker became increasingly concerned about the nature of affliction. Eventually the mad doctor, Dr Willis was called for, also known as Dr Duplicate as he had twin degrees in both theology and medicine. The King's madness had a major influence on the course of psychology, all of which is wonderfully reviewed in Ref. 6. Scene taken from *The Madness of King George*, with kind permission from Renaissance Films (1995).

of photosensitization; and (3) mixed porphyria, where both acute symptoms and skin lesions are apparent.

The neurovisceral dysfunction found with the acute porphyrias is associated probably with either the accumulation of the early intermediates ALA and porphobilinogen (PBG) or a deficiency of haem in neural tissue[7,11]. The photosensitization is a result of the accumulation in tissues of porphyrins that can react with molecular oxygen and light to produce damaging free-radical species. The symptoms of photosensitization include burning pain, itching, skin fragility with blisters and scarring, pigmentation and increased hair growth.

The evidence for porphyria

In looking at the problems allied with George III, Macalpine and Hunter initially suggested that the King suffered with acute intermittent porphyria (AIP)[1]. They even found evidence that the King produced discoloured urine (described at various times as bilious, deep coloured, bluish and bloody; see Fig. 3), which was considered significant, as urine from patients with acute porphyria can be dark-red or brown-black owing to the presence of excess porphyrins and oxidised pyrrole species. The investigators presented their evidence as a paper in the *British Medical Journal* in 1966 (Ref. 2). The discovery and their ideas caused a great deal of interest and one of the major points to emerge was that as AIP is a dominant autosomal disorder, several of the King's relatives would be expected also to have suffered the same metabolic impairment. Herein lies an enigma with porphyria; the disease is not fully penetrant, that is, those who carry the defective gene are not necessarily symptomatic, in fact there is an 80% chance that the holder of the gene will be asymptomatic[7]. The reasons for this are unclear, but the genetic and environmental background of the patient would appear to be important. Attacks of porphyria can also be precipitated by drugs (especially sulphonamides and barbiturates), stress, diet, alcohol and hormones.

The family team of Macalpine and Hunter joined forces with Professor Claude Rimington, the distinguished porphyrin biochemist, to embark on a follow-up project[12]. In this, the investigators searched for evidence of the disease in related historical figures and tried to trace the disease to living descendants. In their eyes, absolute proof for their hypothesis could be established only by showing the presence of the disease in living relatives. The first part of the exercise proved to be the simpler. The investigators showed that many historical figures exhibited some symptoms of the disease. These included Mary, Queen of Scots, James I, Queen Anne, Princess Charlotte and even Edward, Duke of Kent (Queen Victoria's father)[6,12] (Fig. 4). The investigators also claimed to have found that several of these historical figures had a certain amount of skin sensitivity, and on this basis, they changed the original diagnosis from AIP to variegate (mixed) porphyria (VP). To imply that all these figures had the disease would require a large scale re-evaluation of history so that the major events, which were associated with their reigns could be put into perspective[13]. Although porphyria is a reasonable differential diagnosis for the illness of George III, and possibly, James I, the evidence in the case of the others is much less convincing and, as noted by Alan Bennett, 'the slightest regal indisposition was seized on to fetch the sufferer under porphyria's umbrella'[14].

Living descendants and aristocratic intrusions

In order to substantiate the porphyria theory, it was absolutely crucial that living family members with the disorder be traced and examined by the most modern available methods. How does one trace living descendants of the Royal houses of Europe and ascertain whether they suffer with a disease that is tainted with madness? Initially the investigators tried to contact family members by sending them a letter together with sample bags for stool and urine collection. Needless to say, they received little cooperation.

Their second tactic was to ask their medical colleagues for the identity of Royal patients who might have suffered from the disorder. This approach proved the more rewarding and they reported two patients, referred to only as A and B, who they felt had symptoms consistent with variegate porphyria[12]. Patient B was reported to them by the Head of the House of Hanover (the Duke of Brunswick) and was described as 'an old girl who visited Hanover regularly and whose maid had noticed the tell-tale red urine colour' (I. Macalpine and C. Rimington, unpublished). They obtained a stool sample from this 'old girl' and found slightly elevated readings, although with hindsight, we now believe that these readings were not diagnostic of porphyria.

They heard about the second patient (Patient A) from a friend of Rimington who said he had 'treated the Princess during the war'. He had found elevated levels of porphobilinogen and coproporphyrin in her urine and had unhesitatingly diagnosed her as porphyric. The problem was that the readings were taken during the war, which had ended twenty years before the communication with Macalpine, and all written records had since been destroyed. Patient A, however, was still alive, but, when approached by Macalpine and Rimington, she refused to cooperate. We can, however, have confidence in the recollection of the physician, the distinguished Dr Vannotti, who had seen and treated Patient A. He was, at the time, a respected authority on porphyria[15], so it is likely that he would accurately remember the details of such an aristocratic patient, even after 20 years. Furthermore, something to which the authors did not allude was that the daughter of Patient A also had symptoms, which could be attributed to porphyria. Again Macalpine and Rimington were hoping for the cooperation of Patient A's daughter, as they knew that a clinical and biochemical diagnosis would strengthen their case, but like her mother, she refused to help (I. Macalpine and C. Rimington, unpublished).

The difficulty in obtaining the help of Europe's foremost aristocratic families cannot be understated and all the efforts of Macalpine and Rimington were strewn with difficulty, even from those, like the Duke of Brunswick, who appeared to cooperate. For instance, Macalpine and Rimington asked for, and received, a urine sample from the Duke. Analysis of this urine sample proved to be 'weakly' positive, and as the Duke was a descendant of George III they took this as evidence that porphyria had passed into this line. However, we now know that the Duke probably procured the urine sample from his first wife, who was totally unrelated to George III (M. J. Warren, J. C. G. Röhl and D. M. Hunt, unpublished).

Despite these problems, Macalpine and her colleagues managed to put together all their information and facts in a second publication on the occurrence of porphyria in the Houses of Prussia, Stuart and Hanover[12] and brought the porphyria debate to the fore. The problem was not so much the content of the paper, but the manner in which it was reasoned. The authors created tensions by writing as though the case were proven, a situation that could easily have been relieved if they had advanced their views as the 'porphyria hypothesis', allowing readers to make up their own minds. Certain members of the scientific community argued strongly against the suggestions laid out[16–18], including Charles Dent and Geoffrey Dean, the physician who discovered that the high prevalence of VP in South Africa could be attributed to a common descent from a founder who emigrated to the Cape from Holland in the 17th century[18]. They were certainly correct to question some of the theories, and underlying their concern was a genuine fear that the story might be accepted 'hook, line and sinker' by historians. Even today, the treatise put forward should be regarded as a hypothesis. It does, at least, offer a reasonable explanation for the symptoms from which the King suffered and the evidence presented by Macalpine, Hunter and Rimington for the occurrence of the disease in the living relatives is tangible, if rather weak.

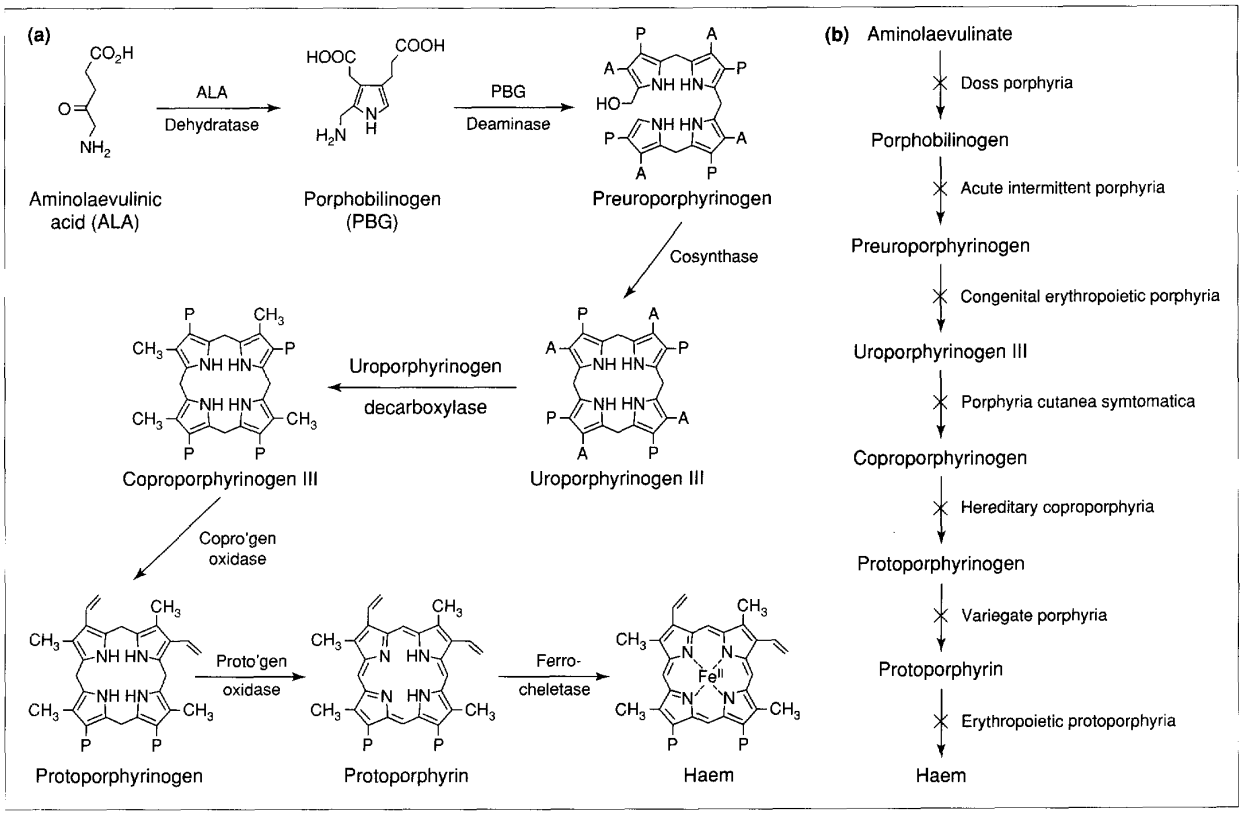

Figure 2

(a) The biosynthesis of haem from 5-aminolaevulinic acid. The first three enzymes in the pathway build the macrocyclic infrastructure, which is subsequently modified and aromatized. **(b)** Porphyrias resulting from lesions in any of the seven enzymes of haem biosynthesis.

Figure 3
A key part in the film was the astute observations made by the King's pages on the colour of his urine, a point that did not interest his physicians. Sufferers of porphyria produce urine that can be discoloured during acute attacks. The pages also noted that the urine returned to its normal colour as the King's health returned. Scene taken from *The Madness of King George*, with kind permission from Renaissance Films (1995).

From American Independence to World War One, porphyria on trial

No further reports of porphyria in members of the houses of Stuart, Hanover or Prussia have been reported in medical journals since 1968. However, one interesting development has been the discovery of a cache of 60 letters written by Princess Charlotte, granddaughter of Queen Victoria and great-great granddaughter of George III. In these letters, Charlotte describes to her doctor, the world-renowned Dr Schweninger, who was also Bismarck's physician, the debilitating conditions from which she suffered. Her ailments included 'agonising abdominal pain, rashes, muscular weakness and the production of red/brown and orange urine'[19]. The reader is left with the strong suspicion that Charlotte indeed might have inherited VP, probably from her mother, Queen Victoria's eldest child. The historical importance of this discovery will be discussed elsewhere, but one point to bear in mind is that Charlotte's brother was Wilhelm II, the last German Emperor. Thus, not only does porphyria remain on trial for its role in the start of the American war of Independence by its influence on the character of King George III, it might now be implicated in influencing the character of Wilhelm II and the start of World War One.

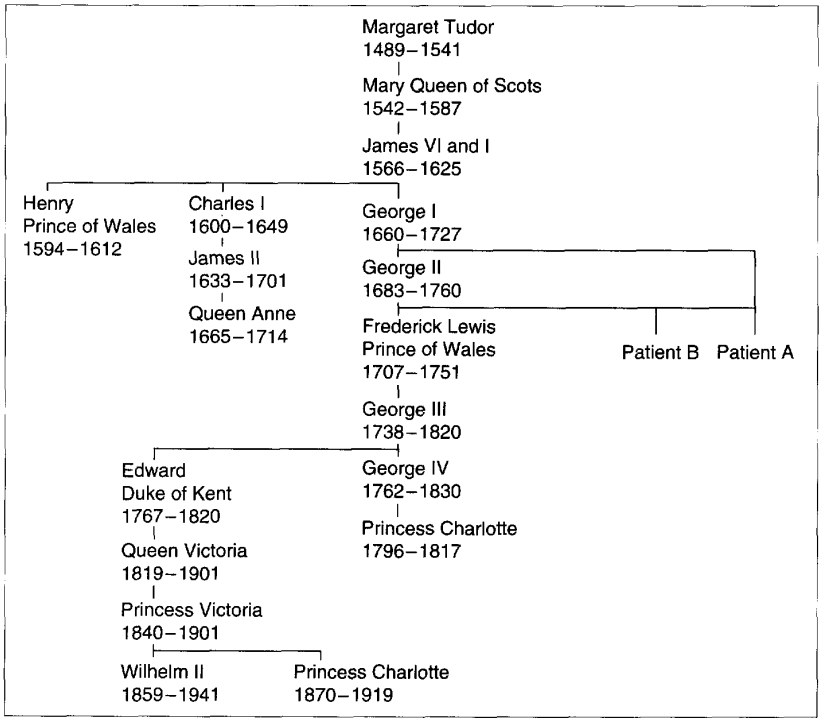

Figure 4
Brief family tree highlighting some of the figures who are thought to have suffered with the Royal porphyria.

Recent advances in the study of the Royal porphyria

What is our current understanding of the porphyrias implicated in the Royal malady? We shall begin by looking at the advances that have been made in understanding the molecular basis of AIP and VP, the two forms of porphyria at the heart of the porphyria hypothesis. All the human genes for haem biosynthesis have been cloned and identified[11,20,21]. The gene for AIP is located on chromosome 11, spanning about 10 kbp and encodes for the enzyme porphobilinogen deaminase[22]. The gene contains two promoters that allow differential expression of two isoenzymes, the erythroid and non-erythroid (housekeeping) forms. The two mature transcripts, which are produced from the gene, differ only in exon 1 and 2, with the housekeeping transcript (exons 1, 3–15) translating into a protein with a molecular mass of 42 kDa, whereas the erythroid transcript (exons 2–15) gives rise to a protein product with a molecular mass of 44 kDa. The subsequent analysis of many AIP-affected families by gene and cDNA sequencing has permitted the identification of over 80 mutations for AIP. Out of these mutations approximately 20% are splice mutations, 10% early terminations, 10% deletions/insertions and 60% amino acid mutations.

Protein studies revealed that porphobilinogen deaminase has evolved a fascinating mechanism whereby the enzyme proceeds through its catalytic cycle via discrete, stable, enzyme-intermediate forms (enzyme with one, two, three and four pyrrole units attached to the active site)[10]. In actual fact, the active site was found to contain two pyrrole units preformed into a co-factor, termed the dipyrromethane co-factor[8]. The *E. coli* enzyme has been overproduced and the purified protein crystallized. The subsequent X-ray diffraction studies allowed the derivation of a three-dimensional model to 1.76 Å resolution, which shows that the polypeptide chain for deaminase is folded into three domains circumventing a large active site crevice[23]. The presence of the co-factor acts as an active-site indicator, pinpointing the exact locality at which the active-site chemistry must occur. This directly led to the identification of Asp84 as a key catalytic group in the enzyme mechanism[24]. The structure also highlighted the role of a number of conserved arginine residues that line the active site.

The high degree of identity between the bacterial and human enzyme permitted

the modelling of the human protein onto the structure of the *E. coli* deaminase. The analysis of around 60 mutations for AIP on this model has given a clearer insight into the molecular basis of AIP[25] (Fig. 5). For example, according to the model, Arg116 makes an important ion pair with Glu250, forming an inter-domain salt bridge. Mutation of either Arg116 to Thr, Trp and Gln or of Glu250 to Lys results in disruption of this important contact leading to loss of protein stabilization and rapid protein breakdown.

Progress on the study of the gene responsible for variegate porphyria has been somewhat slower. This was owing to the difficulty in protein purification with the result that antibodies and protein-derived sequence information were difficult to obtain. Consequently, the first protoporphyrinogen oxidase gene sequence was not reported until 1992 (Ref. 26) and the human gene sequence was reported only late in 1995 (Refs 20, 21). The gene is about 4.7 kb in length, contains 13 exons and encodes a protein with a molecular mass of ~50 kDa. The enzyme requires FAD as a coenzyme and the protein contains a putative dinucleotide-binding site. The gene has been localised to chromosome 1, and evidence that this is the gene for VP has come from linkage studies and the identification of mutations within the protoporphyrinogen oxidase gene in patients with VP (Refs 21, 27).

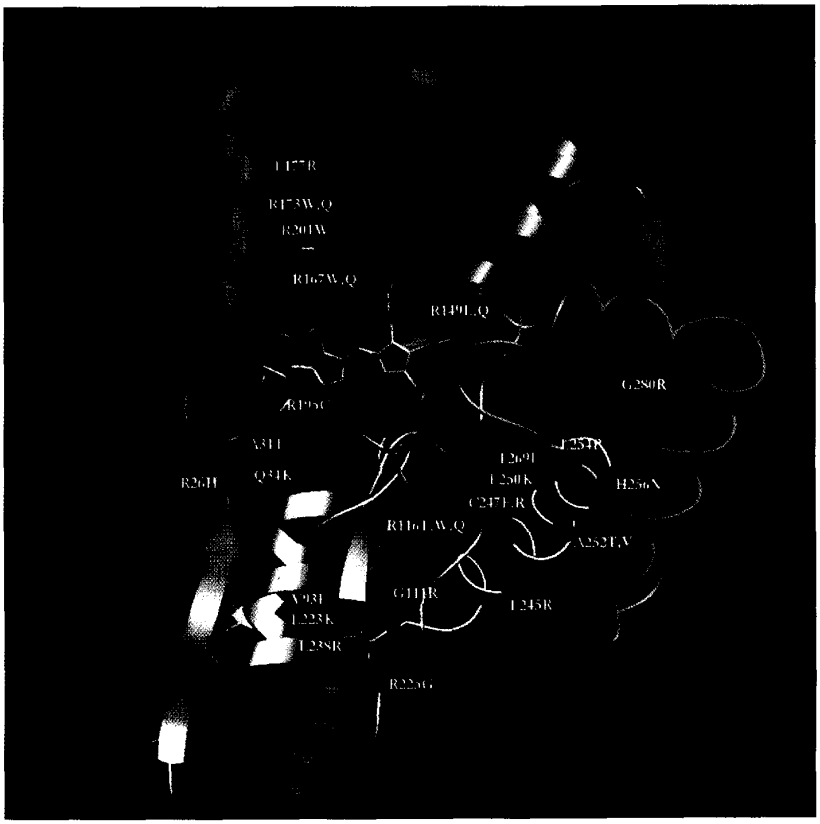

Figure 5
Molecular model of human phorbobilinogen deaminase highlighting some of the known mutations that cause acute intermittent porphyria. Could George III have suffered from such a mutation?

Retrospective diagnoses

Did King George III suffer with AIP or VP? Is it possible to make a posthumous diagnosis using modern technology? The first successful retrospective diagnosis of a genetic disorder in a historical character was the identification of the molecular basis of colour blindness in the famous 19th century chemist John Dalton[28]. While very much aware of his colour blindness (he gave the first authoritative account of this condition in a lecture to the Manchester Literary and Philosophical Society in 1794), he incorrectly proposed that his visual deficiency was due to the presence of a blue filter in his vitreous humour. On his death in 1824, his eyes were removed for examination, but no filter was found. The tissue was not discarded, but was allowed to air-dry and now resides in the Manchester Museum of Science and Industry. With the development of techniques to study ancient DNA, it became possible to amplify DNA from John Dalton's eye tissue and show that he was a dichromat, specifically a deuteranope, who lacked the middlewave photopigment of the retina. A similar molecular biographical approach might help provide an answer to the porphyria hypothesis, if suitable material can be uncovered.

In conclusion

The occurrence of porphyria in the Royal Houses of Europe still remains unproven. The porphyria hypothesis, as it should be called, has provided an interesting debate for the last 30 years. The story enlivens lectures on haem and tetrapyrrole synthesis and makes students think of the implications of a small change, not only on protein form and function, but also on wider issues relating to modern history. The theory has of course been brilliantly adapted by Alan Bennett in his hugely successful stage and film production, *The Madness of King George*, the former even containing a role for Dr Ida Macalpine to explain to the King's hand servants, who were the only people to question the discoloured excrement, the significance of the King's blue urine.

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Getting the message across

Signal Transduction Protocols (Methods in Molecular Biology, Vol. 41)

*edited by **David A. Kendall** and **Stephen J. Hill**, Humana Press Inc., 1995. US$64.50 (xi + 305 pages) ISBN 0 89603 298 1*

The area of signal transduction has taken quantum leaps in terms of its development over the last decade or so, and the various techniques, methods and assays used to address this particular aspect of cell biology have increased accordingly. Consequently, there now exists a myriad of books containing this particular type of format covering all disciplines of biological sciences. Some of them prove to be useful, for example, Sambrook, Fritsch and Maniatis, and some of them are not so useful. *Signal Transduction Protocols*, edited by David Kendall and Stephen Hill, falls into the useful category.

The editors have chosen the contributors well, who in turn have provided detailed protocols for a range of topics concerning receptor-mediated cell signalling. The chapters are short, though not restrictively so, and with only two exceptions, follow a simple format: a brief 'Introduction', followed by the 'Materials' required to carry out a successful experiment, followed by the 'Methods' in point form, followed by a 'Notes' and a 'Reference' section. The two exceptions are chapters one and 24 covering radio-ligand binding and techniques for the measurement of nitric oxide, respectively. These chapters are more descriptive in their format in comparison with the other chapters, but nevertheless, adequately assess the methods currently in use in these areas of research.

Of particular interest in the remaining chapters are the notes sections accompanying each chapter. These sections appear quite beneficial in the majority of cases. They list the typical practical and artifactual problems usually associated with such assays, and advise caution, when required, in the interpretation of the results. As with the notes sections, the materials and the methods sections are also well written and not short on the detail necessary for the reader to be able to conduct the experiments: phrases such as '...put on the lid...' and 'Connect the power supply leads to the electrodes...' give the reader a feeling of security, safe in the knowledge that the author has covered every angle of the most likely pitfalls that might be experienced in the course of the procedure in question. Should the reader require further details, comprehensive reference sections follow each chapter.

The book can be loosely dissected into two major themes of interest. The first is that of the cAMP/cGMP signal transduction pathways. Several chapters are presented covering adenylate cyclase activity, cGMP formation, mass measurements of cAMP and its level in biological membranes. In addition, chapters covering the activity of enzymes intimately involved with regulation of these cyclic nucleotides, i.e. cAMP-dependent protein kinase and cyclic nucleotide phosphodiesterase isoenzymes, are also addressed.

The second theme is that of the inositide–Ca^{2+} signalling pathway and the various players involved in this aspect of signal transduction. Separation of inositol phosphates by high-performance liquid chromatography (HPLC), assays for phospholipase C, D and A_2 are all covered, in addition to the mass measurements of several key intermediates in inositide metabolism, for example PtdIns(4,5)P_2, Ins(1,4,5)P_3, Ins(1,3,4,5)P_4 and diacylglycerol. Three chapters on the methods currently in use to measure intracellular Ca^{2+} concentrations, fluxes and Ca^{2+} imaging are included, and as with the cAMP/cGMP story, the inositide–Ca^{2+} theme is rounded off by the inclusion of two chapters describing the enzymes Ca^{2+}–calmodulin-dependent protein kinase and protein kinase C; two well established enzymes that are activated by the second messengers produced in these pathways.

Finally, there is an assortment of miscellaneous chapters that appear peripheral to the main body of the book. Radio-ligand binding, *in situ* hybridization and a chapter on nitric oxide measurement – given that guanylate cyclase is stimulated by nitric oxide this is a nice inclusion – are all treated with the same degree of detailed coverage as the other chapters.

It is always difficult assessing how much or how little information is to be included in publications of this kind. David Kendall and Stephen Hill have done well to restrict not only the subject matter to well linked themes, but also to restrict the authors to providing a detailed summary of one particular methodology. Of course, there are always variables to consider when dealing with these types of methods, for example, which isoform, which tissue and so forth, but this is the job of the researcher to optimize the conditions to suit him or herself. A recommended book for laboratories dabbling full time with these aspects of signal transduction.

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