

# The Discovery of Dent's Disease: A Personal Account

by

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I was lucky enough to work alongside Professor Oliver Wrong (1925-2012)<sup>1</sup> at University College Hospital (UCH), London, in the 1980s. Oliver - though it took me about 20 years to get on first-name-terms with him - was one of only a few professors of medicine in the Medical School at that time. His interest was nephrology, specifically a large group of very rare diseases of the renal tubule.

The tubule is the part of kidney which, once blood has been filtered by the glomerulus - the ball-shaped network of capillaries that does the kidney's key work of filtering the blood - reabsorbs most of the water and salts back into the body. Once blood has gone through the glomerulus, the tubule fine-tunes the chemical content of urine to keep body composition fairly constant in terms of water and salts. Think of marathon running in a hot climate, or the large salt intake after eating a double-cheeseburger, to grasp what extremes the kidney must cope with.

The sorts of diseases Oliver studied are so rare that a general nephrologist might see only one or two in a lifetime. But Oliver saw hundreds of patients, each of whom had a rare tubular disease or disorder.

Oliver was highly academic and was very highly-regarded for his earlier discoveries about how man adjusts the acidity of the body<sup>2</sup>. He was also a world-expert on nephrocalcinosis, 'NC', an unusual pattern of calcium depositing in the kidney and often found in those rare patients who cannot adjust the acidity of their bodies.

Little was known about how NC was caused - this is still the case. It was the finding of NC, visible on a simple X-ray, which first alerted Oliver to one patient, found many years later to have what we now call 'Dent's Disease'. To complicate things, patients with Dent's disease may also be affected by very much more common 'ordinary' kidney stones often seen in men; they rarely affect the function of the kidney but cause a great deal of pain. (I have had one!). When these very rare kidney conditions occur, they can have a serious effect on bone, particularly in children. The bone disease often found in patients with NC and Dent's disease has the general descriptor of 'metabolic bone disease', a catch-all term which, for example, includes a special type of rickets. This is different to the more common type of rickets due to lack of Vitamin D. The links between kidney and bone disease fascinated Oliver, as did the very unusual patterns of kidney stones in many of his patients.

Before graduating in medicine at UCH I had completed a PhD in biochemical genetics at the University of California at San Diego, working on some of the rare disorders of 'gangliosides', important molecules in cell membranes<sup>3</sup>. My supervisor was Prof. John S. O'Brien, co-discoverer of the enzyme defect which causes Tay-Sachs disease. This meant I was familiar enough with human genetics to recognize patterns created by diseases which run in families. Although my day job was in the routine chemical pathology department at UCH, run by Professor "Freddy" Flynn, we had funding for a small research project. The project was

aimed at finding out why only some patients with bone marrow cancers, usually 'myeloma', whose immune system often produce large amounts of abnormal proteins, develop severe kidney disease.

We made measurements on a type of urine protein, 'Bence-Jones protein', named after the scientist who first discovered it in the 19<sup>th</sup> century, Henry Bence-Jones F.R.S. (1813-1873). The technique we used to look at these Bence-Jones proteins was borrowed from neuroimmunology and uniquely allowed the accurate direct measurement of a physical characteristic of the Bence-Jones proteins known as the 'pI'<sup>4</sup>. The idea (hypothesis), quite widely held at the time, was that whether or not a patient with myeloma developed kidney disease was related to the pI of the Bence-Jones protein. The work was helped by funding I received for a superb part-time colleague, Leah Fulcher. Leah and I did a lot of the bench-work together on the 'pI hypothesis' and later on Dent's disease, but I was coming to the realisation that the original hypothesis was not correct and the research was not really going anywhere.

The technique we were using to measure pI had an interesting 'spin-off'. It was very good at detecting the normal proteins in urine related to Bence-Jones proteins; these proteins are called 'polyclonal light chains' (LC), and we all excrete tiny amounts of these in urine. The kidney works in a strange way. Although the 'glomeruli' in the kidney filter large quantities of these proteins from the blood, most of them are then reabsorbed back into the blood by the renal tubule and broken down to amino acids for reuse. The specialized part of the tubule which does this is the 'proximal tubule' or 'PT'. This process of filtering very large quantities of chemicals also goes on for body water and salts, as mentioned above. Several rare diseases which affect the PT were discovered in the early 1900s and in patients affected by these diseases, normal reabsorption of water, salts and proteins such as the LCs stops or is much reduced. Clinicians had begun to realise that measuring LCs and other proteins of low molecular weight in urine was an excellent way to see how well the PT was working.

Freddy Flynn was one of the first to discover that this disease of the PT left a 'protein signature' in urine of LCs and other low molecular weight proteins. In 1958 he and his future wife, Liz Butler, published a key paper reporting this<sup>5</sup>. So we knew in the early '80s that finding this protein signature, involving LCs but also another protein, Beta-2-microglobulin (B2m), was a red flag signalling PT disease. B2m was easy to measure in minute amounts in normal urine and when the PT was damaged, the increase in quantity in urine was massive, a thousand-times. Both B2m and the LCs belong to the family of urine proteins termed 'Low Molecular Weight Proteins', or LMWP. This was a gift to the clinicians who needed to know how well the PT was working in their patients.

Because I realised that the pI hypothesis of kidney damage in myeloma was flagging, I looked around for other useful applications of our technique. Oliver, like most nephrologists at that time, had a very close relationship with the chemical laboratory doing blood and urine analyses and Freddy introduced me to him. They were very different in their scientific approaches but there was clear mutual respect and no grand-standing. The stage was set to use the pI technique to look at the urine of many of the patients whom Oliver saw in his highly specialist clinic, which ran on Monday mornings. He would typically turn up late lunch-time bearing gifts of urine samples for me to analyse.

I remember when he handed me the first sample from a patient whom we now know to have had Dent's disease. The urine bottle would be labelled with a brief clinical account of the patient as well as their I.D. So the labels would tend to get completely overwritten by Oliver's scribbles. What stuck in my memory is that he mentioned as he handed over the sample that an uncle in the same family had a similar pattern of NC and calcium stones in his kidneys and that in both patients the kidneys were 'not working well'.

It is important but not straightforward (!) to have an idea of what doctors mean if kidneys are said "not to be working well". As mentioned, the kidneys change what is excreted in urine to keep body composition fairly constant. We are subjected to large changes in water and salt intake during the day – think marathon running, starvation or the double-cheeseburger. The kidney's flexibility allows the body to accommodate all this. Clinicians usually describe how well the kidney works in terms of one number; this is the glomerular filtration rate or GFR, usually established via a simple blood test. So generally, if a clinician says that the kidneys 'are not working well', it means that GFR is low: less blood than normal is being filtered by the kidneys over a day. But GFR does not tell the whole story. It does not tell one directly about how the PT, or a number of other parts of the kidney are working. This is particularly important in relation to Dent's disease, since patients, as we later discovered, usually have reduced PT function many years before there is any change in the GFR number measuring rate of blood filtration. So there is no simple link between PT function and GFR. In general the decreased filtration and lower GFR occur in early adulthood in patients with Dent's disease though there are no hard and fast rules.

Oliver eventually saw the patient's uncle in his clinic and noticed two striking similarities between uncle and nephew. They both had the same unusual pattern of NC and bone disease and I found they both excreted massive amounts of LCs in their urine. We confirmed this by accurate measurement of B2m, another LMWP, in their urine. This combination of protein abnormalities is conveniently known as 'Low Molecular Weight Proteinuria'.

The stage was now set for Oliver to track down other members of this family and for me to measure LCs and B2m in many other patients in his clinic with unusual kidney stones. Collecting urine specimens from members of the families involved often meant trips to the English countryside so that the two of us could collect urine and blood samples from family members. We started to draw up our first 'family-tree' of Dent's disease, a simple diagram to show what pattern of characteristics, such as NC and urine LMWP, appeared in each family member. This might provide a clue as to whether the disease, if it indeed was a single disease (a big 'if' at that stage) was inherited.

We found that Dent's patients, even before any drop in GFR, had a number of other consistent abnormalities in their urine as well as LMWP. They excreted too much water and calcium<sup>6</sup>. Proteins in urine are often unstable, so accurate measurement is impossible after the urine has been kept at room temperature, but we found that they usually remained intact long enough for urine samples to be returned in the ordinary post. This was a real help in getting samples from individuals scattered over a wide geographic area. We would send out urine pots and special prepaid envelopes for patients, and other members of their families, to return these samples.

Just because two members of a family have a similar disease, does not mean it is hereditary. Chance may play a big part; researchers must ask themselves: "What are the odds?" We needed a clue about heredity and that was helped by studying the urine of the mothers of the boys and men who had the rare combination of NC, bone disease and LMWP. When we found our first mother who had definite LMWP it was confirmation that the disease was likely to be hereditary. This lady did not have all the features of Dent's disease (nor did she develop these later) but she did have one definite sign of it, LMWP, though much less pronounced than in her son or brother. It also made it more likely that this was an 'X-linked' disease, i.e. a disease like the better-known haemophilia that is usually passed on by females who themselves could be unaffected or only mildly affected.

Using the clinical findings of NC and bone disease, and the laboratory findings of LMWP and increased excretion of calcium in the urine, Oliver identified about 7 families with Dent's disease<sup>6,7</sup>. I visited many of these patients to make sure we were getting accurate 24 hour collections of urine; this was critical to ensure we had reliable measurements for calcium excretion. The pieces of the jigsaw puzzle were beginning to fit together.

Why did so many of these patients excrete an increased amount of calcium in urine? Oliver thought it possible that the underlying problem in these patients was too much absorption of calcium by the gut and that this in turn caused the NC. This would mean that Dent's disease was primarily a gut and not a kidney disease. This may seem a fine distinction but it is critical and it was one which Oliver and I batted back and forth for a long time. Definitive proof of a kidney origin for Dent's disease was to come much later.

When the first families were discovered, Oliver and I requested a meeting with an eminent professor in a closely related field - we told him we thought we might have found a previously unidentified disease. What was memorable was his indifference towards the findings in these families, which were dismissed as 'non-specific', likely random and unconnected signs of other known diseases. Oliver and I were a bit upset about this at the time, but his towering intellect saw both of us through this episode.

Oliver's intellectual leaps on the topic of Dent's disease still amaze me. There were so many false trails which he deftly ignored and I went down. But even the clinical and laboratory descriptions left things incomplete and were far from conclusive proof that this really was a 'new' disease and that it was genetic and X-linked-

For that proof Oliver turned to molecular genetics<sup>8</sup>. The group led by Professor Rajesh Thakker, now a Fellow of the Royal Society (F.R.S.), and then at Northwick Park Hospital and the Royal Postgraduate Medical School in England, showed definitively that Dent's disease was caused by a mutation on the X-chromosome.

In a collaboration with Ian Craig and Thakker, a gene termed *CLCN5*, which encoded a specialist kidney-specific 'chloride-ion channel' known as CLC-5, was identified and shown to have mutations in patients with Dent's disease<sup>9,10</sup>. Chloride channels such as CLC-5 facilitate the movement of the chloride ion into and out of cells. There are many different types and they were originally discovered by Professor Thomas Jentsch's group of the Max Delbrück Centre in Berlin, Germany. This places Jentsch's work at the heart of just how defects in

CLC-5 cause Dent's disease and there is on-going research in several centres world-wide to understand this.

Takashi Igarashi at the University of Tokyo in Japan, along with Thakker, also established that children with the 'Japanese' disease which had up until then been labelled 'idiopathic low molecular weight proteinuria' in fact had Dent's disease<sup>11</sup>. They were actually the same disease. This showed the global importance of Oliver's work. These and other stories form a vital continuum with Oliver's insights. Photograph #1 includes Thomas Jentsch alongside several others involved in the discoveries of Dent's disease.

I have given a personal account of Dent's disease but it has ignored the fact that what we know as Dent's disease Type1 (Dent's 1) was 'discovered' around the same time by some 5 other groups! The group led by Professor Steve Scheinman found a very large family<sup>12</sup> affected by it, and Scheinman was a major collaborator with Wrong and Thakker in identifying the Dent's 1 chloride-channel gene mutation<sup>10</sup>. The Scheinman group was also instrumental in identifying a mutation which causes a related but different disease, Dent's disease Type2<sup>13</sup>.

There is a figure missing from my story so far and that, of course, is Professor Charles Dent F.R.S. (1911-1976)<sup>14</sup>, pictured in photograph #2, after whom the disease was posthumously named. Dent trained as a pure chemist, later became medically qualified and at U.C.H. became a pioneer of what became known as 'metabolic medicine'. It was Oliver's personal choice, with the agreement of Charles Dent's family, to name the disease 'Dent's disease'<sup>6,7</sup>.

How and why did Oliver choose the name and what part does Professor Dent actually play in this story?

Naming this 'disease' at all was actually quite a leap, since at that stage it was done without a proper molecular genetic description. It was quite possible that many of the findings were indeed 'nonspecific', as suggested by Oliver's sceptical eminent professorial colleague. At least a year after identification of the first family with Dent's disease, Oliver told me about a report by Charles Dent and Max Friedman which had been published in 1964<sup>15</sup>. I am sure neither of us had seen it before then, since we always discussed intensively anything published about diseases with these kinds of characteristics.

In their paper, Dent and Friedman described two unrelated patients with LMWP, metabolic bone disease and increased excretion of calcium in their urine. One of these patients had mild mental retardation and the other did not. Dent and Friedman did not identify any hereditary cause for the disease in these two patients. In fact they thought it was probably not hereditary and speculated that the two patients might have had 'heavy-metal poisoning'. Cadmium salts, sometimes absorbed by workers involved in cadmium plating of iron or making batteries, were well known 'heavy-metal' poisons of the kidney tubule.

Later work by Professor Thakker's group showed that one of the two patients reported by Dent and Friedman was, in fact, affected by a mutation in the *CLCN5* gene<sup>10</sup>. Professor Scheinman's group discovered that the other patient originally described by Dent and Friedman, with mild mental retardation, had a mutation in a quite different gene, '*OCRL*'<sup>13</sup>. This has led to the identification of Dent's Disease Type1 and Dent's Disease Type 2. Type 1

patients, who are in the majority, are defined by the *CLCN5* gene mutation and Type 2 patients by the *OCRL* mutation.

This brings an extraordinary twist to the whole story and links Professors Dent, Wrong, Scheinman and Thakker. Professionally, Oliver and Charles Dent had only a slight connection and I suspect that describing Dent as Oliver's 'mentor', as is sometimes done, may be misleading. However, Oliver did succeed Charles Dent as Professor of Medicine at UCH. If Oliver had a 'mentor', it was more probably Professor Alexander Leaf at the Massachusetts General Hospital, who became a close friend, but I say that based on personal anecdote. Dent was one of the pioneers of the study of human metabolic hereditary diseases. Oliver, I think, named the disease, which Oliver himself had discovered, as 'Dent's disease' for two reasons: there was the scientific thread with Dent and U.C.H., London, but also the name was pithy, memorable and suitable as an easily recognisable 'brand name'. 'Wrong's Disease', as Oliver himself told me, could have led to all sorts of potential mix-ups and practical problems.



Photograph #1. Six individuals intimately involved in the discovery and early work on Dent's disease. From left to right:- Professors Rajesh Thakker, F.R.S; Oliver Wrong; Steven Scheinman; Thomas Jentsch; Terence Feest and Dr. Anthony Norden (author of this account). The photograph was taken at a meeting titled 'A Half-Century of Renal Tubular Disease, a Symposium in Honour of Professor Oliver M. Wrong', held in London in March 2009.



Photograph #2. Professor Charles E. Dent, F.R.S., Professor of Human Metabolism at University College Hospital, London from 1956-1976.

There are probably other mutations, yet to be discovered, which will complement the two types of Dent's disease which we now know. But as I hope this story makes plain, it was Oliver Wrong who blazed this particular trail. The major contributions of at least 5 other groups, which Oliver freely acknowledged<sup>7</sup>, deserve their own stories.

This account may seem to ignore the personal impact of Dent's disease on those affected and their families. These were actually very close to Oliver's heart. Well after his official retirement from Britain's National Health Service, members of the families he'd followed would call him at home asking for medical advice for sons and grandsons they feared might be affected by the disease. He fretted continuously about the well-being of all his patients. It is hoped that, notwithstanding the distress this disease causes, Dent's disease patients and their families can find a place to honour Oliver Wrong.



*Author's Note:* This is a personal account and not a formal scientific review. The reference list is deliberately kept short and is not at all comprehensive. Several points have been simplified and use of specialised scientific terms such as 'renal Fanconi syndrome' omitted. Also the vital role of abnormal phosphate handling in this disease has, for example, been left out for clarity. Urine acidification defects are not mentioned. The precise chloride ion transport system affected in Dent's disease has not been discussed. Forgive me for not citing the many medical scientists who gave advice about these patients at the time. Drs. David Brenton and Marta Lapsley, Prof. Robert Unwin at UCH, London, Prof. Martin Barratt C.B.E. (1936-2014) and William van't Hoff at the Institute of Child Health, London and Alessandro Mutti from Parma, Italy are among these but this list is incomplete. Many biomedical scientists at UCH undertook careful measurements on the urine of patients with Dent's disease in the 1980s. The Wellcome Trust in London maintains an archive of the original laboratory and clinical papers of both Oliver Wrong and Charles Dent (<https://wellcomecollection.org/works/vj3jeavw>). This archive contains numerous primary materials relating to Dent's disease from Oliver Wrong, many colleagues world-wide and this author. I am very grateful to Prof. Raj Thakker for reviewing an early draft of this account. Prof. Steve Scheinman made several helpful suggestions. Michela Wrong, a daughter of Oliver Wrong, kindly made valuable revisions. Of course, any errors or inaccuracies remain my responsibility.

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