Darwin diagnosed?

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Abstract

While waiting in lodgings to join HMS Beagle just before Christmas 1831, Charles Darwin suffered chest pain and heart palpitations. On his return to England he began to suffer from a range of gut problems, and systemic symptoms around the body, which were to plague him for the rest of his life. At least forty conditions have been proposed to explain Darwin's illness, which left him disabled, sometimes for weeks on end. Here we show that lactose and food intolerance is the only condition that explains all his symptoms. Furthermore, there is now a molecular basis to account for these, based on metabolic toxins produced by microbes in the intestine. This mechanism has important implications in several other diseases, including diabetes, inflammatory bowel disease, Parkinson's disease and some cancers. Lactose intolerance also has fascinating things to tell us about molecular evolution - the origin of lactose, the unique sugar in milk; why white humans were able to invade the plains of Europe after the last ice thaw, some 10,000 years ago; and one of the most intriguing problems in evolution – the origin of a new enzyme such as lactase, the enzyme responsible for cleaving lactose into its constituents monosaccharides, galactose and glucose.

Introduction

On the 1st July 1858 Charles Lyell and Joseph Hooker presented two documents from Charles Darwin and one from Alfred Russel Wallace, to a meeting of the Linnean Society in London, entitled 'On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties and Species by Natural Means of Selection'. Neither Darwin nor Wallace was present to enjoy this momentous event, though its importance was apparently unrecognised by the President and audience at the time. Wallace was still in the Malay Archipelago. But Darwin was unable to attend because his sickly baby son, Charles Waring, had died of scarlet fever two days earlier on Tuesday 29th June (Burkhardt & Smith, 1991a). He and Emma were too distraught. Yet it was his own illness that usually prevented Darwin taking his full part in the British scientific scene. Five years after the Linnean Society meeting, Charles Darwin, according to Emma's diary (Darwin, (1824 - 1896)), had vomited every day since mid-November 1863, and wrote in a letter to his friend Joseph Hooker, Director of Kew, on 5th December 1863, (Burkhardt, Porter, Dean, Topham & Wilmot, 1999):

'I have had a bad spell, vomiting every day for eleven days, & some days many times after every meal'.

For over 40 years Darwin (Fig. 1) suffered from a debilitating illness, which was never diagnosed or cured (Bowlby, 1990; Campbell & Matthews, 2005a; Colp, 1977, 2006; Dixon & Radick, 2009; Hayman, 2009b). His illness is documented in over 400 of his letters (Burkhardt, Porter, Dean & al., 1989 - 2015), in his Diary of Health written from 1st July 1849 and 16th January 1855 (Colp, 2006; Darwin, 1849-1855), his autobiography (Darwin, 1876; Darwin, 1902), and Emma's own personal diaries (Darwin, (1824 - 1896)). It often left him disabled for weeks on end. He wrote to his friend Joseph Hooker (1817 – 1911) on 1st Sept 1859: 'I had a terrible long fit of vomiting yesterday, which makes the world rather extra gloomy today.' It was his illness that prevented him attending the famous meeting in Oxford of the British Association for the Advancement of Science 27th June – 4th July, 1860, when Huxley had his famous altercation with Bishop Samuel Wilberforce. On 25th June, 1860. Darwin wrote to Charles Lyell (Burkhardt, Porter, Browne & Richmond, 1993a):

I have given up Oxford; for my stomach has utterly broken down & I am forced to go on Thursday for a little water-cure, to Dr Lane at Sudbrook Park, Richmond, Surrey, where I shall go for a week, and sh.d stay longer if it had not been for Etty (his daughter Henrietta Emma, who had been ill since April).

Then on 2nd July, 1860, from his friend Joseph D. Hooker, Darwin received news about the successful defence at Oxford of his hypothesis about Natural Selection (Burkhardt, Porter, Browne & Richmond, 1993b). This cheered Darwin up, who was still ill at Sudbrook Park, writing back on 2 July, 1860 (Burkhardt, Porter, Browne & Richmond, 1993c):

I have just received your letter. I have been very poorly of late with almost continuous bad headache for 48 hours, & I was low enough & thinking what a useless burthen I was to myself & all others, when your letter came & it has so cheered me up. Your kindness & affection brought tears into my eyes'.

Over 40 years, he saw some 20 doctors (Colp, 1977, 2006; Hunting, 2009), including his father, many of whom prescribed a range of medications. None had any beneficial effect. Indeed several made him feel worse. , Here we show that all the symptoms that Darwin suffered from can be explained by lactose intolerance. Furthermore, there is a molecular basis for these symptoms, which have important implications for several conditions commonly seen by general practitioners and medical specialists today. Several books have been published which describe his illness and possible causes (Bowlby, 1990; Colp, 1977, 2006; Pickering, 1974a). A wide range of conditions have been proposed (Table 1), many focussing on just a few particular symptoms. But none, until now, explain **all** of his symptoms.

The natural history of Darwin's illness

Darwin survived scarlet fever when he was nine, and, at the age of 20, noted excessive fatigue, suffering from mouth sores and eczema while at Christ's College, Cambridge. But his first major problem arose while waiting in lodgings to join HMS Beagle just before Christmas 1831. Charles Darwin suffered chest pain and severe heart palpitations. Fearing he had had a heart attack Darwin told no one, lest he was not allowed on his voyage of a lifetime. He only admitted to this in his autobiography (Darwin, 1876; Darwin, 1902).

'On 25 October, I took up my residence at Plymouth, and remained there until 27 December when the Beagle finally left the shore of England for her circumnavigation of the world. These two months at Plymouth were the most miserable which I have ever spent, though I exerted myself in various ways. I was out of sprits at the thought of leaving my family and friends for so long a time, and the weather seemed to me inexpressively gloomy. I was also troubled with palpitations and pain about the heart, and like many a young ignorant man, especially with a smattering of medical knowledge, was convinced that I had heart disease. I did not consult a doctor, as I fully expected to hear the verdict that I was not fit for the voyage, and I was resolved to go at all hazards.'

He then spent nearly five years on HMS Beagle as naturalist and companion to Captain Fitz Roy. During this time he suffered some illness, particularly seasickness. In the first letter to his Father, Robert Darwin, he wrote about 'The misery I endure from seasickness'. His Beagle diary records a few other problems, such as severe inflammation of knee, and then arm, while in Brazil from March – July 1832, which lasted a week. He records 'Feverish, shivering and sickness with exhaustion and loss of appetite,' and on 11 April 1832; feeling 'Very weak from great heat, unwell and feverish from too much sun. On 2nd October 1832, he was unwell and feverish, and in bed, on two following days; and then 'not quite well, stomach disordered.' While at Montevideo on 16 Oct 1833; and then in Chile again from 20th September to the end of October 1834 he had a bad stomach, felt exhausted, and suffered from a high fever in bed, which was possibly typhoid. He ascribed this to drinking chichi (*chicha*) – a fermented grape juice in a gold mine he visited, and owned by a Mr Nixon. Thus, although his seasickness has been linked to a diagnosis of cyclic vomiting syndrome when back in England (Hayman, 2009b), he did not appear to suffer from the wide range of both gut and systemic symptoms from which he suffered the rest of his life.

His son Francis (1848 – 1925) wrote:

'For nearly forty years he never knew one day of the health of ordinary men, and his life was one long struggle against weariness of strain and sickness'.

Some have argued that Darwin failed to turn up to certain events because he was afraid to face his critics. In fact, it was his illness. It was this that prevented him attending the famous Oxford debate in 1860 (Burkhardt et al., 1993a), when Bishop Samuel Wilberforce was confronted so successfully by T H Huxley (1825 – 1895). Following Darwin's return in 1836, he started to work on his Beagle material and notes, marrying his cousin Emma Wedgwood on 29th January 1839. They began married life in a house in Gower Street, in central London, which they named Macaw cottage, and where they lived until 1842. But, in 1837 he had written to one of his Cambridge mentors, John Henslow, who had obtained his invitation to travel on the Beagle some six years earlier, stating he was having regular heart palpitations, eczema, erythema, and headaches. As a result he stopped going to parties. The air in Victorian London was hardly healthy. So Charles and Emma sought a place in the country, where he could concentrate on his work, and hopefully benefit from the clean, country air. In September, 1842 they moved to Down House, in the village of Down(e) in Kent, a house that remained in the Darwin family until 1906. Although Darwin's literary output from Down House was prodigious, including many scientific papers, some 15,000 letters, and 25 books, he suffered from some twenty gut and systemic symptoms for the rest of his life, though he appears to have been slightly better during his last few years. He died of heart failure and angina on April 19th 1882 (Colp, 2006). He recorded most of his symptoms in a Diary of Health between 1 July, 1849 to 16 January, 1855, growing a permanent beard in 1860 after one major bout of ill health, and, in particular, to hide his facial eczema (atopic dermatitis).

His lsymptoms were (Colp, 2006; Colp, 1977; Darwin, 1876; Hayman, 2013; Hayman, 2009):

- Chronic fatigue
- Severe gut problems – pain, belching, flatulence
- Nausea and vomiting
- Severe headaches
- Swimming head, dizziness
- Visual disturbances
- Skin rashes and boils
- Mouth ulcers, tooth and gum problems
- Joint pain
- Heart palpitations
- Chest pain
- Numbness and tingling in the fingers
- Flushing and swelling of his face and extremities
- Lumbago

Sweating

- Insomnia
- Trembling
- Hot and cold attacks
- Depression, acute anxiety, and hysterical sobbing

Later in life, Darwin suffered more serious symptoms, associated with epilepsy, stroke and heart failure (Colp, 2006; Colp, 1977). Over forty causes have been proposed to explain his illness (Table 1). They fall into two main categories – psychosomatic and organic. Psychosomatic causes include bereavement syndrome, hyperventilation and panic attack, and depression (Bowlby, 1956, 1984, 1990). Darwin's mother, Susannah, died when he was just eight years old, and he certainly was depressed at times. Well most people would be depressed if they suffered from all these symptoms, which were never successfully treated. However, it is clear that the majority of his symptoms must have had an organic origin. Darwin's illness was not psychosomatic, in spite of claims that he has been accused of hypochondriac tendencies (Bowlby, 1990; Pickering, 1974). Darwin was aware of some activities that could trigger his illness, even certain stresses, including visits from friends. But he was not able to pin down the real cause. Organic causes proposed have included arsenic poisoning (Winslow, 1971), and Chagas' disease (Adler, 1959, 1965, 1989a, 1990). Also proposed is Ménière's spectrum disorder, as Darwin did have tinnitus, often described as 'ringing in the ears', vertigo, dizziness, motion sickness, seen when at sea, vomiting, and fatigue. But his full symptoms do not fit Ménière's disease itself (Gordon, 2009a, b). Other conditions proposed include various gut ailments, such as cyclic vomiting syndrome, and Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS), involving an inherited mitochondrial mutation (Finsterer & Hayman, 2014; Hayman, 2013b), irritable bowel syndrome (IBS) (Shanahan, 2012), and Crohn's disease (Orrego & Quintana, 2007). His regular bouts of vomiting certainly fit the diagnosis of cyclic vomiting syndrome (Hayman, 2013; Hayman, 2009), which is associated with nausea, abdominal pain and dyspepsia. But, this does not explain all the other symptoms Darwin suffered from. Furthermore, cyclic vomiting is a clinical description, and not a mechanism. The molecular basis of nausea and sea-sickness involves dopamine receptors. Whereas cyclic vomiting syndrome is caused by endocrine dysfunction, classic sea-sickness is caused by a conflict between signals to the brain from the eyes or balance organs, such as the ears. Thus, many people who suffer seas-sickness do not have cyclic vomiting syndrome. However, cyclic vomiting syndrome can also be associated with mood changes, migraine and severe headaches, fatigue, anxiety, and dizziness (Kumar, Bashar, Reddy, Sengupta, Ananthakrishnan, Schroeder, Hogan & Venkatesan, 2012; Lee, Abbott, Mahlangu, Moodie & Anderson, 2012; Yang, 2010). But, in spite of the attractive diagnosis of cyclic vomiting syndrome, this, and other proposed conditions do not explain **all** Darwin's symptoms, or their timingacutely and long-term. We propose that the only condition that does is lactose and food intolerance (Campbell and Matthews, 2005), the latter involving intolerance to foods other than milk, such as bread and fruit, and ant foods containing particular sugars, such as fructose. Typically, patients with lactose intolerance produce a lot of flatus, and often have diarrhoea. But, a substantial number suffer from constipation, not diarrhoea (Table 3) (Matthews, Waud, Roberts & Campbell, 2005; Waud, Matthews & Campbell, 2008).

Arsenic poisoning can occur in two phases - acute with nausea, vomiting, tachycardia, diarrhoea, breathing difficulties, and chronic with melanosis, keratosis, gangrene, skin cancers over 10 years, and internal cancers over 20 years. These do not match Darwin's symptoms. Although it is possible that Darwin was exposed in Argentina to water contaminated with arsenic, his father, a doctor, was not keen on it as a medicine. So Darwin mostly avoided it.

A popular diagnosis has been Chagas' disease (South American trypanosomiasis) (Adler, 1959, 1989b, 1990; Bernstein, 1984; Goldstein, 1989), first described by a Brazilian doctor, Carlos Chagas in 1909. It affects more than 150 animal species, including some 7-8 million humans in Mexico, Central and South America, and also in the USA. It is caused by the protozoan *Trypanosoma cruzi*. This trypanosome, like *Giardia*, infects the small intestine and other organs, and thus would be expected to cause gut problems, such as lactose intolerance. It is now also known that post-infection from *Giardia* can leave the patient with intra- and extra- intestinal problems, including irritable bowel syndrome, chronic fatigue, cognitive difficulties, and growth deficiency (Halliez & Buret, 2013; Naess, Nyland, Hausken, Follestad & Nyland, 2012).

A well-known carrier of *Trypanosoma cruzi* is the great black bug of the Pampas. This is a member of the Triatominae, a subfamily of Reduviidae. They are commonly known as conenose bugs, kissing bugs, assassin bugs, or triatomines. The 'kissing bug' term arises from the fact that they often attack the lips or eyelids. They are haematophagous, i.e. they feed on vertebrate blood, though a very few species feed on other invertebrates (Sandoval, Duarte, Gutierrez, Rocha, Angulo, Esteban, Reyes, Jurberg & Galvao, 2004; Sandoval, Joya, Gutierez & Angulo, 2000), and are widespread in the Americas, with a few species present in Asia, Africa, and Australia. A typical wide spread bug is *Triatoma infestans*, known as 'vinchuca' in Argentina and Chile, or 'barbeiro' in Brazil. Darwin was attacked by one he called 'Benchuca', and one of the officers on the Beagle kept one as a pet. Darwin describes it after sucking blood;
'This one feast, for which the Benchuca was indebted to one of the officers, kept it fat during four whole months; but, after the first fortnight, the insect was quite ready to have another suck'.

Darwin noted in his journal for March 24th 1835, in a village 5 leagues south of Mendoza, Argentina;
'We crossed the Luxan, which is a river of considerable size, though its course towards the sea-coast is very imperfectly known. It is even doubtful whether, in passing over the plains, it is evaporated, or whether it forms a tributary of the Sauce or Colorado. We slept in the village, which is a small place surrounded by gardens, and forms the most southern part, that is cultivated, of the province of Mendoza; it is five leagues south of the capital. At night I experienced an attack, & it deserves no less a name, of the Benchuca, the great black bug of the Pampas. It is most disgusting to feel soft wingless insects, about an inch long, crawling over ones body; before sucking they are quite thin, but afterwards round & bloated with blood, & in this state they are easily squashed.'

However, the trypanosome is not transmitted by the bite, but rather in the faeces. Nevertheless, when one is bitten by one, the tendency is to brush it away, when it defaecates and this infects the wound. Chagas' disease occurs in three stages: an acute stage which can last 4-8 weeks, involving a fever, eye swelling, large liver and spleen, enlarged lymph glands, fatigue, rash, appetite loss, diarrhoea and vomiting; an intermediate stage, which can last anything from 8 weeks to a few years, with no obvious symptoms; and a chronic stage, which can last 10 – 40 years, involving heart failure, gastro-intestinal problems, swallowing problems, and severe constipation. So the real problem with this diagnosis is that again the Chagas' disease does not explain **all** Darwin's symptoms, nor the timing of either the acute or ling term symptoms. This is important when comparing Darwin's symptoms to those of lactose intolerance. A further important point is that trypanosomiasis is different from Giardiasis, and can infect the smooth muscle of the intestine. Thus, trypanosomiasis is less likely to cause lactose intolerance, which is invariably associated with gut infection such as Giardiasis and gut viral infections.

An attractive diagnosis is Crohn's disease (syndrome) (Ortega and Quintana 2007), first described at Mount Sinai Hospital in New York in 1932 by a gastroenterologist Burrill Bernard Crohn, and two colleagues, involving inflammation of the terminal ileum of the small intestine, and is a form of inflammatory bowel disease (IBD). It can affect any part of the GI tract, from the mouth to the anus, but the most common digestive tract conditions are ileal, ileocolonic (45%), and colonic. Symptoms include abdominal pain, diarrhoea with blood, fever and weight loss, but patients can also suffer some systemic symptoms, such as anaemia, skin rashes, eye inflammation, fatigue, headache, depression, and seizures. The patient gets better when the gut is better. Crohn's disease is now categorised via the Montreal classification (Satsangi, Silverberg, Vermeire & Colombel, 2006), and diagnosis is confirmed by a combination of radiology, colonoscopy, and biopsy, to show inflammation and granulomas. Some risk genes have been found associated with this syndrome, an important one being NOD-2 (Barthel, Spalinger, Brunner, Lang, Fried, Rogler & Scharl, 2014; Burstein, Szilagyi & Smith, 2010; Hugot, Chamaillard, Zouali, Lesage, Cezard, Belaiche, Almer, Tysk, O'Morain, Gassull, Binder, Finkel, Cortot, Modigliani, Laurent-Puig, Gower-Rousseau, Macry, Colombel, Sahbatou & Thomas, 2001; Liu & Anderson, 2014; Ogura, Bonen, Inohara, Nicolae, Chen, Ramos, Britton, Moran, Karaliuskas, Duerr, Achkar, Brant, Bayless, Kirschner, Hanauer, Nunez & Cho, 2001), a significant protein in the immune system, which activates the NFκB transcription pathway (Strober, Asano, Fuss, Kitani & Watanabe, 2014), the protein's full name being nucleotide-binding oligomerisation domain-containing protein 2 (NOD-2) or caspase recruitment domain-containing protein 15 (CARD15), and is found on chromosome 16. NOD-2 has 1040 amino acids divided into four main domains – 2 CARD, 1 NOF, and 1 leucine rich (Fig. 2). The leucine rich domain is a receptor for the bacterial product muramyl dipeptide. There are three common mutations in NOD-2 – R702W, G908R, and L1007 frame shift, as well as 27 other rare mutations. Mutations tend to result in decreased NFκB activity, which could lead to problems with the gut immune system, thereby leading to gut inflammation. Importantly, heterozygotes with mutations in NOD-2 can have a two fold increased risk of Crohn's, and homozygotes a twenty fold increased risk. However, these mutations are not diagnostic. But, although some of Darwin's symptoms appear to match those of Crohn's disease, it is very difficult to see how he could have had a long standing Crohn's disease affecting the upper GI tract, since it would have been fatal in Darwin's day. Furthermore, a diagnosis of Crohn's does not explain Darwin's most troubling symptoms of episodic nausea, vomiting and flatulence, and Crohn's disease is 'not sufficient for subsuming his pleiomorphic symptomatology' (Sheehan et al, 2008). Interestingly, although there is no correlation between lactase persistance and NOD-2 (Elguezabal, Chamorro, Molina, Garrido, Izeta, Rodrigo & Juste, 2012), lactose intolerance is found in a significant number of patients with IBD, and thus Crohn's disease (Eadala, Waud, Matthews, Green & Campbell, 2008).

Another gut related condition suggested for Darwin's illness is irritable bowel syndrome (IBS) (Shanahan, 2012), one of the most common conditions seen by general practitioners and gastroenterologists. The most common gut symptoms are abdominal pain and cramping, a change in bowel habit, bloating and swelling of the abdomen, and an urgent need to go to the toilet. What is not well known is that patients with IBS or IBD also exhibit systemic symptoms (Matthews, Waud, Roberts & Campbell, 2005; Waud, Matthews & Campbell, 2008). Furthermore, as many as 70-80% of these patients are sensitive to lactose. They are lactose intolerant.

But, even if Darwin had been suffering from Chagas' disease, IBD, IBS, or lactose and food intolerance, there is little chance that the twenty doctors who examined him would have been able to make such a diagnosis. First, none of these conditions were known in Darwin's day. Secondly, the modern techniques of radiology, endoscopy, and histological examination of biopsies were not available until well into the 20th century.

In spite of a lack of diagnosis, Darwin was subjected to a range of therapies, including what we would regard as poisons, such as arsenic, calomel ($HgCl_2$) and bismuth. For example, the medicines prescribed by Dr Henry Holland and Dr James Clark included bitters, mineral acids, alkalies, pepsin, tonics, phosphated iron, calomel, Cody's ozonised water, arsenic (small doses), and tartar emetic ointment for eczema. On the other hand, Darwin's father, in November 1840, recommended logwood, from the tree *Haematoxylum campechianum,* used to treat chronic diarrhoea, dysentery, and haemorrhages from uterus, lungs, or bowels. Darwin's father hoped it would improve his son's appetite and digestion. He also prescribed potassium bicarbonate for gastric acid, but was not normally in favour of arsenic. A further possible therapy was from his late grandfather, and given to his sick daughter Annie – bread and milk (Keynes, 2001), a major problem for anyone suffering from lactose intolerance. Darwin's own remedy may have been snuff, of which he was a regular indulger.

None of these medicines were effective. In fact the only treatment which appears to have had any beneficial effect on Darwin was a 'water cure' at Malvern, and first recommended to Darwin by his friend, a second officer on the Beagle, B J Sulivan, and his cousin William James Fox.

The water cure

Between 1849 and 1860, Darwin visited four establishments, which offered the 'water cure' – Malvern, in Worcestershire; Moor Park at Farnham, in Surrey; Ilkley in Yorkshire; and Sudbrook Park, Richmond in Surrey (Burkhardt et al., 1993a; Dixon & Radick, 2009; Grierson, 1998; Gully, 1847; Jenkins, 1972). The water cure facility at Malvern had been set up by Dr James Wilson and Dr James Manby Gully. Famous people who visited there included Alfred Lord Tennyson and Florence Nightingale, strengthening Darwin's confidence in the 'cure'. It was Dr Gully who looked after Darwin. His water cure involved 'involved the application of cold water by baths and 'wet sheets' and dietary restrictions, such as little milk, and no buttermilk, and little snuff. As Darwin wrote; 'At no time must I eat sugar, butter, spices, bacon, or anything good. He allowed me a little milk'. Amazingly, on his first visit, Darwin took the whole family, with servants,from Down House, including Emma, his then six children, the butler and maids. They rented The Lodge at Great Malvern from March to June, 1849, at the time the town centre of Malvern.

In his autobiography p 117 he wrote (Darwin, 1876):
'In October, 1846 I began to work on Cirripedia. When on the coast of Chile I found a most curious form, which burrowed into the shells of Concholepas......Although I was employed during eight years on this work, yet I record in my diary that about two years out of this time was lost to illness. On this account I went in 1848 for some months to Malvern for hydropathic treatment, which did me much good, so on my return I was able to resume work. So much was I out of health that when my dear father dies on 13 November, 1847, I was unable to attend his funeral or to act as one of his executors.'

Thus generally, the water cure made him feel better, so long as he ate and drank at Gully's facility, rather than eating with Emma and the family. But his last visit to Malvern in 1851 ended in tragedy. He had taken his beloved daughter Annie there, as she had been ill be some time, suffering at least some of the 'Darwin symptoms', though some have claimed she had tuberculosis (Fenner, Egger & Gagneux, 2009). On 24th March 1851 she died. Darwin came home to Down House devastated, and could never return to Malvern.

So he next tried a hydropathic establishment at Moor Park, Farnham, set up by Dr Edward Wickstead Lane. He certainly got better there, but his vomiting returned when he went back to Down House. Thus, in July 1859 Darwin was not so good, and decided he could not stay at Moor House, as Dr Lane had been sited in a messy divorce case. So he looked for another water cure centre, though Darwin did return to Dr Lane, who had bought Sudbrook Park, Richmond, in 1860 having left Moor Park (cited in Burkhardt et al., 1993a). Thus Darwin, in 1859, visited Wells House, in the village of Ilkley in Yorkshire, built three years earlier. The hydropathy centre there had been set up by Dr Edmund Smith. Darwin wrote initially that 'Dr Smith I think is sensible, but is a Homeopathist'. However, Darwin began to dislike him, writing 'he cared very much for the fee and very little for the patient'. As with scientists today, Darwin was never convinced about the efficacy of increasing dilutions in therapeutic doses of medicines, even though he appears to have tried successfully this approach on insectivorous plants (Ullman, 2010). Darwin stayed at Ilkley from 4th October to 7th December 1859. One day turned out to be one of the most important in the history of science, for, on November 25th 1859, 'On the Origin of Species' was published. Furthermore, Darwin had very important correspondence there, both before and after publication, which would have been impossible if he had not felt better. Emma came for a while, when they stayed in rented accommodation. Dr Smith was not happy that Darwin was not all the time at Wells House, where he could be exposed to all the full rigours of his water treatment. Once again, while with Emma he was ill, but at Wells House he was better.

Without Emma, he invited a Mary Butler, reputed for here witty conversation, whom he had met at Moor House, to join him. As his son Francis tells us, clearly Darwin had a weakness for younger, attractive women (Darwin, 1902). He enjoyed the company of the ladies! He also enjoyed playing billiards at Wells House, bringing out his competitive nature, and writing enthusiastically about the game to his son William.

The water cure involved cold showers and baths, being wrapped up in wet linen, and drinking lots of spa water (Dixon & Radick, 2009; Grierson, 1998; Gully, 1847). The diet of the water cure included soup, fish, meat and animal products, and vegetables, but only a few condiments. Drinks included water, barley and rice water, weak black tea, but only sometimes milk. Buttermilk (full of lactose), and all puddings, were prohibited. Thus the water therapy diet had very little milk or sugar, compared to his usual meals.

Darwin's correspondence while at Ilkley is of great interest (Table 2) (Burkhardt & Smith, 1991b), particularly as it is further evidence that his health had improved, enabling him to correspond effectively. Letters received included several enthusiastic ones from relatives, friends and colleagues about 'On the Origin', including one from his cousin Francis Galton (Burkhardt & Smith, 1991a). There were several letters to and from John Murray, his publisher, concerning the title, the print run and the price. Once 'On the Origin' was published, Darwin sent complimentary copies to his friends and colleagues, as well as many important people, including the author Charles Kingsley. Interesting scientific recipients included Louis Agassiz, John Herschel, John Henslow and Adam Sedgwick at Cambridge, Joseph Hooker, Charles Lyell, Asa Gray at Harvard (his letter to Gray was part of the presentation to the Linnean Society in 1858), Richard Owen, who first used the word dinosaur and set up the Natural History Museum in London, Alfred Russel(l) Wallace, and the reverend Leonard Jenyns, nephew of John Henslow, who had first been offered the Beagle trip, but had turned it down as he had just been appointed to a parish, and had helped Darwin with his fish specimens from the Beagle (Jenyns, 1842). His friends, Hooker and Huxley, and Wallace, loved the book, but one of Darwin's mentors, Adam Sedgwick, was very critical, and was not keen on the universal concept of Natural Selection, nor its potential conflicts with religious beliefs. The correspondence with Charles Lyell (1797 – 1875) is particularly important, including eleven from Darwin. Darwin wrote in one letter to Lyell; 'I believe Natural Selection will account for probably any vertebrate animal'. But, at first, Lyell, while admiring greatly the scholarship in 'On the Origin', was sceptical about Natural Selection. However, after this lengthy correspondence Lyell seemed eventually to be convinced, though, like Wallace, remained unconvinced that this alone could explain the emergence of humans.

Thus, on 7th December, 1859, Darwin left Ilkley, and returned back home to Down House, via London to see his brother Erasmus. Whatever the reason, the water cure always did him good. In 1850 he had written in a letter to his sister Susan: 'Sickness gradually decreasing and he felt restored'. Then on 6th May, 1858, writing to his friend Joseph Hooker, Director of Kew; 'As usual Hydropathy has made a man of me for a short time.' And then while at Ilkley, in a letter to his eldest son William October 1859 Darwin tells him: 'The Water Cure has done me much good'. And to Hooker on 15th Oct 1859 he says: 'I am hydropathising & coming to life again after having finished my accursed book, which would have been easy for anyone else, but half killed me.'

At Down House Darwin continued taking the water-cure, when he afflicted by an attack of ill-health. He even erected a cold shower in an out-building there. So the question arises, was Darwin's illness one of these so-called multifactorial medical problems, or can one mechanism really explain all of his symptoms, and why did he get better whenever he took the 'water therapy'? What was unknown to Darwin was that lactose and food intolerance, via metabolic toxins produced by microbes in the gut, can indeed explain this.

Lactose intolerance and a molecular mechanism

Any single explanation of Darwin's illness has to cope with four problems:

- The wide range of organ problems that affected Darwin (gut, heart, brain, muscles, joints, immune system, skin, mouth).
- There was no obvious trigger that set off an attack.
- The spasmodic nature and unpredictability of his symptoms.
- Why he got better with the hydrotherapy treatment of Drs Gully, Lane and Smith.

Two of Darwin's remarks point to the solution:
'The sickness starts usually about two hours after a meal'.

And then following a 'water cure':
'Thanks also my Father for his medical advice – I have been very well since Friday, nearly as well as the first fortnight & am in heart about the non-sugar plan'.

Typically, food, not fully digested or absorbed in the small intestine, reaches the large intestine some 2-4 hours after ingestion. Any sugar, particularly lactose - the sugar in milk - that reaches the microbes in the large intestine will be metabolised, producing gases, - hydrogen, methane and hydrogen sulphide - and a range of metabolic toxins (Campbell et al, 2010). Thus acute effects of lactose intolerance occur within a few hours of ingesting lactose. Darwin suffered a number of skin lesions (Hayman, 2011). Such longer term effects, such as skin lesions, boils and eczema, do occur in people with lactose intolerance (Grimbacher, Peters & Peter, 1997; Matthews et al., 2005).

Lactose (Mol. wt 342.3) is only found in large amounts in mammalian milk, cows milk containing about 47 $g.l^{-1}$ (139 mM), and human milk some 70 $g.l^{-1}$ (204 mM). Lactose is a disaccharide, β-D-galactopyranosyl-(1→4)-D-glucose or β galactose 1,4 glucose for short (Fig. 3), being composed of galactose and glucose. Disaccharides, such as lactose, cannot normally be absorbed directly, but have to be broken down into its constituent mono-saccharides in the small intestine by enzymes on the villi. In the case of lactose, this is lactase-phlorizin hydrolase (E.C. 3.2.62 & 108). This enzyme is unique in having two Enzyme Commission (E.C.) numbers, because it has two active centres within the same enzyme, one hydrolysing lactose, the other a diabetogenic substance originally found in apple bark, phlorizin (Fig. 3). In fact, this site has evolved to hydrolyse cerebrosides, and thus supply essential lipids, such as sphingosine, needed for membranes, such as myelin, which enables mammalian nerves to transmit action potentials properly.

The lactase gene is some 70kb long, including the 5' and 3' ends, 55kb containing the 17 coding exons, and is found on the anti-parallel strand on the long arm of chromosome 2 in humans (2q21). The human enzyme, when first formed, has 1927 amino acids(218kDa). There are 10 linked N-glycosylation sites.The enzyme is also processed through proteolysis. leaving the final enzyme with 1059 amino acids, with a large, 1014 amino acid extracellular domain, and a the carboxy terminus and the two active centres facing out into the gut lumen, a 26 amino acid transmembrane domain, and a short, 19 amino acid intracellular domain, with the N-terminus facing into the cytosol. The enzyme forms a dimer in the membrane, with a molecular weight 320,000 Da. From sequence similarities within the protein, it appears that the full protein has been formed during evolution via gene duplications at 87-172, 363-8464, 883-1365, and 1370-1841. Phlorizin binds to both active centres, being hydrolysed at one, and inhibiting lactase activity at the other. Cells expressing the enzyme are formed continuously in the crypts of the villi of the small intestine (Fig. 4). Lactase starts being expressed in the duodenum, reaches a peak in the jejunum, and then gradually decreases down the ileum. Lactase is not found in the large intestine, and is quite distinct from the bacterial and fungal enzyme β galactosidase (E.C. 3.2.1.23), which also hydrolyses lactose into galactose and glucose. There are no amino acid sequence similarities between the two enzymes. In fact, health food shops that claim to sell lactase, are in fact selling β galactosidase. Thus mammals only have two enzymes that can hydrolyse

β-galactosides, lactase in the small intestine, and β-galactosidase in bacteria in the large intestine. Once the lactase is hydrolysed, then the galactose and glucose are taken up in the enterocyte cells via the sodium activated glucose transported SGLUT1 (Fig. 4). This is quite distinct from GLUT5, which transports fructose into these cells. The sugars are the released into the blood via the transporter GLUT2.

'After weaning, mammals do not normally consume milk. So, in evolutionary terms, there was no need to keep large amounts of lactase in the small intestine in adult mammals, just a little to hydrolyse the cerebrosides. Thus, all mammals, except most white Northern Europeans, and some special races such as the Bedouins and certain tribes in Africa, start to lose lactase after weaning. Chinese youngsters may have lost some 95% of their lactase by the age of 5 or 6. Other Asians similarly lose > 80% by teenage. The small number of white Northern Europeans, who lose lactase naturally, will have a low level of lactase by late teenage (Campbell, Jenkins-Waud & Matthews, 2005/2009; Campbell & Matthews, 2005b; Campbell, Waud & Matthews, 2009). This means that some 4000 million people around the world cannot digest lactose properly. Thus everyone can digest some lactose, but a large number of people are sensitive to lactose, because they are hypolactasic (low lactase). But their sensitivity varies considerably. Some can take a litre of milk without any problems, yet others can be sensitive to just a few millilitres.

There are six reasons for having a low lactase level of lactase:

1. Congenital, which is very rare
2. Inherited loss after weaning, which is common
3. Gut infections
    a. Viral; e.g. rotavirus
    b. Bacteria in the small intestine
    c. Protozoal; e.g. Giardia, Trypanosoma
4. Gut damage; e.g. radiotherapy as part of cancer therapy
5. Hormonal imbalance
    a. Sex hormones (menstrual, menopause)
    b. Thyroid over-activity
    c. Ageing
6. Problems with transcription of the gene or enzyme processing

It is important to realise that data on the levels of lactase in the small intestine are based on a small biopsy, usually from the duodenum or jejunum during an endoscopy. It has been estimated that if the cells in the villi of the whole of the small intestine were laid out as single cell layer, then it would cover an area of a tennis court, or even larger. Thus taking a biopsy, to assess the full capacity of lactase in the small intestine to remove all the lactose before it reaches the large intestine, is like taking a few blades of grass from the Millennium Rugby pitch in Cardiff to assess whether it is fit to play an International game!

The first two mechanisms for lactase loss are irreversible, but the others should be reversible, with the right therapy. People who have a low lactase suffer a range of gut and systemic symptoms if they cross their individual lactose threshold (Table 3). Hippocrates, in the 4th century B.C., noted that people in the south of Europe had problems after drinking milk. But it was not until the 20th century that the condition of lactose intolerance was fully recognised, well after Darwin's death. Furthermore, it is only recently that the full extent of the systemic symptoms, such as headaches, fatigue, exacerbation of allergies and heart palpitations, has been recognised in lactose intolerance, irritable bowel syndrome and inflammatory bowel disease (Campbell et al., 2005/2009; Matthews & Campbell, 2000; Matthews et al., 2005; Waud et al., 2008). In fact, in the evolution of humans, dairying is very recent, beginning less than 8000 years ago. Pictorial and written records show that, although cattle, sheep and goats were domesticated in the Near East between 9000 and 7000 B.C., there is no evidence they were milked. However, records from the Sahara region, Egypt and Mesopotamia shows that dairying had begun by 4000 to 2900 B.C. (Dudd & Evershed, 1998; Simoons, 1979). It is interesting to hypothesise that the mutation which allowed the ancestors of white Northern Europeans to keep lactase after weaning, allowed them to increase their calcium intake, and avoid the effects of vitamin D deficiency (Simoons, 2001). It also allowed them to move with their milk-producing cattle into the plains of Europe after the last major ice thaw some 10,000 years ago.

Sensitivity to lactose is quite distinct from an allergy to one or more of the major proteins in milk. Such an allergy is seen in some 3-5% of babies, most of whom recover completely by adulthood. This allergy is immune based, and causes symptoms, such as diarrhoea or constipation, which can overlap with those of food intolerance, but can be separated from the majority of those with lactose intolerance.

A molecular mechanism

There are three reasons for incomplete digestion of lactose in the small intestine: a low level of expression of lactase, inhibition of the enzyme lactase, or, more importantly, inhibition of the galactose and glucose transporter, SGLUT1. The latter can be caused by the tri-saccharide raffinose and the tetra-saccharide stachyose (Fig. 3). These sugars are found at significant levels in many vegetables, such as the root vegetables, parsnips, Jerusalem artichokes, soya and turnips, as well as beans and lentils (Campbell & Matthews, 2005b). It is well known that eating these, with starch, results in the production of a large amount of gas from the intestine (flatus), and thus bloating and pain. This is because blocking of mono-saccharide uptake in the small intestine, allows them to reach microbes in the large intestine. There are some 400 species of bacteria, and two archaeans, in the human large intestine, there being some 10^{14} microbial cells, ten times as many cells as eukaryotic cells in the rest of the body. Here, there is very little oxygen, so the products of sugar metabolism by pathways such as glycolysis cannot be 'burnt' to CO_2 and H_2O, via the mitochondria. So, in order to continue to make ATP anaerobically, it is necessary to get rid of the hydrogen generated in NADH (Campbell, 2010; Campbell, Waud & Matthews, 2005; Campbell et al., 2009). Mammalian muscle does this via the enzyme lactase dehydrogenase generating L-lactate. Some bacteria make either L- or D-lactate, and several bacterial species have a formate hydrogenase, discovered by Marjorie Stephenson in the 1930s, which generates hydrogen gas, the main gas in flatus. The archaeans can use this hydrogen to reduce substrates such as CO_2 or acetate to methane. Archaea are the third domain of life, quite distinct in their biochemistry and molecular biology from eukaryotes and bacteria, and were originally discovered as extremophiles, being able to live at high temperature, high salt, and high or low pH, because of glycerol ethers in their cell membranes, instead of glycerol esters in other cell types. But, archaeans have now been found at other sites. For example, it is the pigment in the archaean *Haloferax volcanii*, which is the cause of the familiar pink colour in flamingos. Importantly. *Methanobrevibacter* species have been found in the human gut, vagina and teeth, the latter being potentially involved in periodontitis. Neither bacteria nor eukaryotes can generate methane. Interestingly, patients diagnosed with inflammatory bowel disease who produce only methane in their flatus had the most severe symptoms (Eadala, 2011).

Importantly, bacteria, and possibly archaeans, also generate a variety of metabolites, particularly alcohols, diols, ketones and aldehydes, in order to get rid of the hydrogen equivalents from glycolysis. These include ethanol, acetaldehyde, butan 2,3 diol, propan 1,3 diol, acetoin, and diacetyl (Campbell, 2010; Campbell et al., 2005, 2009). A particularly important metabolite is methylglyoxal (CH_3COCHO) which can be generated from the glycolytic products glyceraldehyde 3 phosphate and dihydoxyacetone phosphate by both bacterial cells and hepatocytes. The metabolites can affect both host eukaryotic cells and gut bacteria. They can activate apoptosis, provoke smooth muscle contraction in the gut, can affect contraction in the heart, and activate potassium channels, thereby affecting membrane potential (Campbell, 2010; Campbell, Matthews, Vassel, Cox, Naseem, Chaichi, Holland, Green & Wann, 2010; Campbell et al., 2005, 2009). Thus, the effects on heart, brain, immune system, muscles and other tissues can be explained through an effect on cell signalling, in particular the universal intracellular calcium signalling system (Campbell, 2015), which provokes all muscle contractions, nerves firing, exocrine and endocrine secretion, vision, hearing and other senses, activation of cells in the immune system, and egg fertilisation. In bacteria, these metabolites can also induce signals in intracellular calcium (Campbell, Naseem, Wann, Holland & Matthews, 2007; Campbell, Naseern, Holland, Matthews & Wann, 2007), which can activate or inhibit genes (Naseem, Wann, Holland & Campbell, 2009), potentially modifying the balance of microflora in the gut.

Some 25% of patients sensitive to lactose have severe constipation. Methylglyoxal, and potentially other bacterial metabolites, can cause covalent modification of insulin and 5’ hydroxytrypamine (Campbell et al., 2010), the latter via the Pictet-Spenger reaction (Fig. 5). In both cases, this causes inactivation of the hormone or neurotransmitter respectively. In the case of 5HT this would inhibit smooth muscle contraction and thus gut movement. In contrast, inactivation of insulin would be potentially diabetogenic. A further interesting consequence of this microbial metabolic toxin mechanism is that it explains why some 75% of lactose sensitive patients suffer from diarrhoea. This cannot be explained by an osmotic effect of lactose, since patients often suffer from diarrhoea for several days after an initial lactose load, well after the lactose would have disappeared. Thus the microbial metabolic toxins generated in the large intestine, or by microbial over-growth in the small intestine, are absorbed into the blood where they can affect a wide range of tissues and thus cause the systemic symptoms.

A major problem for people who are sensitive to lactose now is that this sugar is added in a variety of forms to a wide range of foods, and drinks, for example in the form of whey from cheese making or milk powder in bread and biscuits. A huge lactose industry has built up since the end of the Second World War, amounting to some 400 million kilogrammes per year in the USA alone. This is then added to human and animals foods. However, this would not have been a problem for Darwin. His only sources of lactose would have been milk and cheese, and cream, the latter containing less lactose per unit volume than full milk.

Nevertheless, the gut and systemic symptoms revealed in patients suffering from sensitivity to lactose (lactose intolerance) match exactly all Darwin’s symptoms (Table

4). There are three ways in which Darwin could have been sensitive to lactose, and other sugars. First, inherited loss of lactase, which does occur in some 10% of white Northern Europeans; secondly, through damage to his gut, via an infection or inflammation; thirdly, though sensitivity to tri- and tetra-saccharides, such as raffinose and stachyose, causing inhibition of sugar uptake via SGLUT1. He could also have had a poor GLUT5, resulting in inefficient uptake of fructose, generated from sucrose hydrolysis. Darwin was known to have a very sweet tooth.

Darwin and DNA

There is a clear family history of illness, including Darwin's symptoms, in the Darwin and Wedgwood dynasties. Charles Darwin's paternal grandmother, Mary Darwin (1740 – 1770), Erasmus' first wife, died aged 30, but had other symptoms, suggesting acute intermittent porphyria (King-Hele, 1968) or tuberculosis (Cook, 1996). Darwin's mother Susannah (née Wedgwood; 1765 – 1817)) had chronic ill health, and died with abdominal pains when Darwin was only 8 years old. His elder brother, Erasmus Alvey Darwin (1804 – 1881) suffered from chronic ill health, including major fatigue and abdominal pain. He never worked, living in London as a socialite and chronic invalid. Darwin's ten children were a sickly lot. Three died young, and Darwin's granddaughter, Gwen Ravarat (1885 – 1957), wrote in her charming book, *Period piece*, 'well known ill health in the Darwin tribe' (Raverat, 1952). Certainly several of his children suffered from the 'Darwin' illness, for example, the two daughters who survived into adulthood. Henrietta Emma Darwin (1843 – 1927) was sickly as a child, but lived to be 84, and Elizabeth Darwin (1847 – 1926) never married. There was also sickness on the Wedgwood side of the family, Josiah Wedgwood (1730 -1795) being Darwin's maternal grandfather. Darwin's uncle, Tom Wedgwood (1771 – 1786), records headaches and abdominal pain since he was a student, and had recurrent chronic fatigue. He died of an opium overdose aged 34. His aunt Mary Anne Wedgwood (1778 - 1786) died as a child. And even Darwin's cousin and wife, Emma née Wedgwood (1808 - 1896), suffered from regular sever headaches, yet also lived to the good age of 86. In fact, several members of the family on the Wedgwood side suffered some of Darwin's symptoms. For example, Darwin's maternal uncle, Tom Wedgwood (1771 – 1805), suffered regular headaches, abdominal pain, and chronic fatigue. He died of an opium overdose at the age of 34. There is even evidence that some of the living descendants of Darwin have lactose intolerance. The diagnosis of lactose and food intolerance would certainly have allayed Darwin's fear that his children's illnesses were due to the problems that can arise from consanguinousmarriages.

The enzyme lactase, with other gut sugar enzymes such as isomaltase and sucrose, are induced just before birth through a major gut homeobox gene, CDX2. However, loss of this transcription factor this cannot be the cause of loss of lactase after weaning, since the other sugar hydrolytic enzymes are retained. What is not generally realised is that loss of an enzyme, such as lactase, may be due to loss of cell number, rather than loss within each cell. Thus a developmental gene is likely to be the cause of lactase loss after weaning. An important developmental event around the time of weaning is the appearance of deciduous teeth. This is activated via the dental homeobox genes BMP-4, MSX-1 and -2, SHH, DLX-1 and -2, and LEF1. The role of these genes in lactase loss needs to be investigated. The only two polymorphisms found associated with lactase loss are in the introns of the gene coding for the helicase MCM6, just upstream from lactase. These are C/T13910 and G/A22018, and have an interesting evolutionary history (Ingram, Mulcare, Itan, Thomas & Swallow, 2009; Potter, Ho, Bolton, Furth, Swallow & Griffiths, 1985; Swallow, 2003). People who are CC and GG are hypolactasic, and all are sensitive to lactose (Campbell et al., 2005, 2009; Matthews et al., 2005; Waud et al., 2008). However, people who are CT/GA or TT/AA can also be sensitive to lactose.

In view of the apparent correlation of Darwin's symptoms with illness on the female side of Darwin's family, it has been proposed that the cause of Darwin's illness was an inherited mitochondrial mutation A3243G in a mitochondrial leucine tRNA gene, which is found in some patients with Mitochondrial Encephalomyopathy, Lactic Acidosis and Stoke-like episodes (MELAS) (Hayman, 2009a, 2013a, 2013b). In humans, mitochondria are inherited entirely through the mother's egg, none surviving from the father's sperm. If Darwin and his family members did have this mutation, it would be necessary to explain how he was sensitive to lactose, and other sugars. This has not been investigated in patients known to have this mutation.

In 2014, a television programme on UK Channel 4 claimed to have a sequence of Darwin's DNA from hair samples known to be his. This has been highly controversial, and must be regarded as anecdotal, as no DNA analysis of Darwin's has been subjected to rigorous peer review. However, the presenter, Professor Schuster, insisted that Darwin had a mutation in a gene coding for a 1040 amino acid Crohn's disease risk protein NOD-2, known also as CARD15. Although we have shown that many patients with inflammatory bowel disease (IBD) – ulcerative colitis or Crohn's disease – were lactose sensitive (Eadala et al., 2008), it is not clear whether this is cause or consequence. Furthermore, there appears no correlation between NOD-2/CARD15 polymorphisms and lactose intolerance (Elguezabal et al, 2012). Risk genes are distinct from causative genes, such as those responsible for cystic fibrosis, sickle cell anaemia, or familial hypercholesterolaemia. There is, at present, little or no evidence that the risk genes identified in many diseases such as cancer, diabetes, asthma and Alzheimer's disease, with lead to the cause to these conditions. In fact a recent study on a large cohort of cancer patients concluded that their problem was as much due to bad luck than bad genes (Tomasetti & Vogelstein, 2015).

Thus lactose and food intolerance is the only condition that explains all Darwin's symptoms. First and foremost, Darwin had an organic illness. He did get depressed, but his symptoms cannot be explained by a psychosomatic condition. He only got better when he reduced his milk intake, and through the water therapy of Dr James Gully at Malvern, at Moor Park, at Ilkley, and at Sudbrook. There would have been little or no fresh milk on the Beagle. He got worse when he was at home eating Emma's cooking. Her recipe book, held at the University Library at Cambridge, shows over 75% of the desserts had milk or cream, regular Béchamel sauces served with the meat course, and root vegetables, that contain sugars inhibiting galactose and glucose uptake in the small intestine, were commonly eaten. Furthermore, although we have no definitive evidence about Darwin's DNA, there is a clear hereditary factor, several of his close ancestors, relatives and children, having many of his symptoms, which appears to be still present in the current generation. Finally, and most importantly, there is a molecular mechanism which can explain the hitherto bewildering diversity of symptoms arising directly from the gut, and around the body (systemic) – microbial metabolic toxins generated by bacteria and Archaea in the large intestine, and microbial overgrowth in the small intestine induced by sugars in this hypoxic environment (Campbell et al., 2010; Campbell et al., 2005, 2009).

Darwin and the evolution of milk

The appearance and evolution of milk, lactose and lactase have much to teach us about Natural Selection, and the evolution of humans. Darwin's output was prodigious, including some 25 books. Many of his books provided evidence to support his big idea, and that of Wallace, of evolution by Natural Selection. But, interestingly, one thing is missing, that clearly distinguishes humans, and all mammals, from every other animal on our planet – the production of milk (Darwin, 1877). But the sugar, lactose, unique to milk, was unknown to him. In fact, the word milk only appears twice in The Descent of Man, and nowhere else in his science, though, of course it is mentioned in his letters with respect to the water cure.

It may, at first, seem surprising that Darwin, and his scientific colleagues, appeared ignorant about lactose. Lactose ($C_{12}H_{22}O_{11}$), derived from the Latin *lac* or *lactis* for milk, was discovered way back in 1619 by Fabrizio Bartoletti (1576 – 1630), and published in 1633 (Bartoletti, 1633). In 1700, Lodovico Testi (1640–1707), a Venetian pharmacist, produced a booklet as a testimonial to the value of milk sugar he called Saccharum (o) Lactis, in relieving arthritis and other ailments (Testi, 1700, 1715). Lactose was recognized as a sugar by Carl Wilhelm Scheele, the co-discoverer of oxygen, in 1780 (Scheele, 1780a, b). Heinrich Vogel (1778-1867) found that glucose was a product of hydrolyzing lactose in 1812 (Vogel, 1812a, 1812b), and in 1856, Louis Pasteur succeeded in crystallizing galactose (Pasteur, 1856), the other component of lactose, galactose. It was one of the most important chemists of the Nineteenth century, Emil Fischer, who established in 1894 the full configurations of the sugars, first from grapes (Fischer, 1891a, b), and then from lactose in 1894 (Fischer & Morrell, 1894) By the early 20th century it was established that the names of sugars such as lactose end in –ose, from the Latin meaning 'full of', 'abounding in', 'given to' or 'like'. In contrast, enzymes, such as lactase, end in –ase, the latter from Duclaux and Buchner (Duclaux, 1899; Buchner, 1907).

Lactose is some one sixth as sweet as sucrose. There is an obvious selective advantage of mammals using lactose in milk, instead of glucose, fructose or sucrose, is that, being not so sweet, it would not attract insects to the mother's nipples. On the other hand, the persistence of lactase after weaning in white Northern Europeans, and some other human groups, is a classic example of 'niche construction', the process by which a population of organisms develop components of their local environment in a way that produces new selections pressures (Gerbault, Liebert, Itan, Powell, Currat, Burger, Swallow & Thomas, 2011). This dominant Mendelian trait has increased to a high frequency in central and Northern Europe during the past 20,000 years, but was rare in Neolithic Europeans, prior to dairying (Burger, Kirchner, Bramanti, Heak & Thomas, 2014). The age origin of the alleles associated with lactase persistence ranges from 1,200 to 23,200 years (Ingram, Elamin, Mulcare, Weale, Tarekegn, Raga, Bekele, Elamin, Thomas, Bradman & Swallow, 2007; Ingram et al., 2009).

Darwin was a very clever man, as was his Father, Robert Darwin, a good doctor. So why didn't he diagnose himself`? During Darwin's lifetime, the science of chemistry made huge strides. But biochemistry was in its infancy in the nineteenth century. Carbohydrate, fat, and protein had been recognised by Justus von Liebig (1803 – 1873) (Brock, 1997), but their significance in the biochemistry of the body only really became apparent during the first quarter of the 20th century. The concept of food intolerance, as opposed to food poisoning, was virtually unknown. Lactose intolerance was not fully recognised until well into the 20th century, and only in the late 20th and early 21st century were the systemic symptoms resulting from lactose sensitivity fully documented (Campbell et al., 2010; Campbell et al., 2005, 2009; Matthews & Campbell, 2000; Matthews et al., 2005; Waud et al., 2008). Furthermore, in Darwin's time, there was no mechanism to explain such a diversity of both gut and systemic symptoms.

Chapter VI in (On) The Origin of Species is entitled 'Difficulties on/of the theory'. These were Darwin's difficulties, in seeing how a new phenomenon, such as the electric organ of a fish or the flashing of a firefly or jelly fish, or even a complex organ such as an eye, could have appeared, and then evolved, small change by small change through Natural Selection. His difficulty now can be seen in the light of modern molecular and cellular biology. A real puzzle is how a new enzyme, such as lactase, or a bioluminescent luciferase, could have appeared, apparently out of the blue. Mammals first appeared from synapsids in the late Carboniferous period (358.9 – 298.9 Ma), there being synapsid like mammals in the mid-Triassic period (252.2 – 201.3 Ma) (Lefèvre, Sharp & Nicholas, 2010). It has been proposed that the selective advantage of milk arose from the need of reptiles and egg-laying mammals (monotremes), who laid soft eggs without a hard calcified shell, to keep them from drying out. So they had to secrete a liquid to keep the eggs moist (Oftedal, 2002a, b). The mammary gland may have evolved from skin or the immune system (Goldman, 2002; Lefèvre et al., 2010; Vorbach, Capecchi & Penninger, 2006). But which came first lactose or lactase? Both involve new enzymes.

Many enzymes have sequence similarities with others, giving suggestions about a common evolutionary origin. But how did an original enzyme, such as lactase, arise, before being susceptible to the forces of Natural Selection? There are equal difficulties in explaining why evolution has chosen just A, T, G, and C as the coding bases in DNA, why ATP not GTP was chosen to drive endergonic reactions, why D-sugars were chosen in nucleic acids and energy carbohydrates, and why L-amino acids were chosen in proteins (Campbell, 1994). In spite of intense research using genetic engineering over the past 40 years, attempts to genuinely generate a new enzyme have failed. Many effects on the properties of an enzyme have been achieved – affinity for substrates and inhibitors, maximum activity, and effects on covalent modification or binding to another protein. But a dehydrogenase is still a dehydrogenase, a kinase is still a kinase, and a luciferase is still a luciferase. There have been examples of a mutation apparently changing an enzyme into a new one. For example, resistance of blowflies to organophosphate insecticides can occur via a G137D mutation in the carboxyesterase, which changes it to an organophosphate hydrolase (Newcomb, Campbell, Ollis, Cheah, Russell & Oakeshott, 1997). However, even in this case, the substrate remains the same, and the enzyme still acts to cleave it. Fred Hoyle highlighted the problem of generating new biological processes by random mutation in his famous analogy with a Jumbo jet (Hoyle, 1981):

'The chance that higher life forms might have emerged in this way is comparable to the chance that a tornado sweeping through a junkyard might assemble a Boeing 747 from the materials therein' (Hoyle, 1981).

The gene coding for lactase in humans produces a protein initially of 1927 amino acids. Thus, with each site having a choice of 20 amino acids, the total number of possibilities by random mutation is 20^{1927}, or 10^{2507}, an impossibly huge number, when the number of stars in the Universe is estimated to be only $9x10^{21}$. Even 4000 million years, the approximate time that life has existed on our planet, is not enough to generate a specific new enzyme. But Hoyle was wrong. Using bioluminescence, the first item described in Darwin's hand written Beagle zoology notebook (Darwin, 1832), we have shown that only a few amino acids forming a pocket, that acts as a solvent cage, binding a particular substrate, is sufficient to generate a new enzyme (Campbell, 2012; Vassel, Cox, Naseem, Morse, Evans, Power, Brancale, Wann & Campbell, 2012).

Outcome of Darwin's illness

So there are three positive outcomes of Darwin's illness. First, if he had not moved to Down House in 1842, where he stayed until his death in 1882, he would not have been able to devote his whole career to writing, microscopical examination, experiments, scholarship, and letter writing, in spite of periods which stopped him working effectively (Johnston, 1901; Pickering, 1974a, b). Darwin did not often go to scientific meetings, and certainly not around the world as we do today. He worked all morning in his study, had lunch, and spent the afternoon answering his letters with Emma. He also had time to carry out experiments in his green house and garden, with his son Francis (Frank). As Darwin himself wrote (Darwin, 1876):

'Lastly, I have had ample leisure from not having to earn my own bread. Even ill-health, though it has annihilated several years of my life, has saved me from the distractions of society and amusement'.

Secondly, the molecular explanation of his symptoms through metabolic toxins generated by gut microbes (Campbell et al., 2010; Campbell et al., 2005, 2009) provides a new approach, not only to lactose and food intolerance, but also to other conditions, such as unexplained heart palpitations, allergies such as eczema, inflammatory bowel disease, irritable bowel syndrome, the type 2 diabetic epidemic, some cancers, such as prostate and breast, where high milk intake appears to be a risk factor, infertility, Alzheimer's and Parkinson's diseases. In fact, when Parkinson first described his syndrome, he pointed out that his patients first suffered gut problems, before exhibiting the well-known shaking.

Thirdly, lactose, and the enzyme that cleaves it, lactase, highlight a fascinating problem in evolution, which worried Darwin – the origin of a new process. Although his diagnosis is over 175 years too late (Le Fanu, 2005), Darwin would no doubt have been delighted that his condition has had such a positive outcome.

In Borneo, there is a pitcher plant, *Nepenthese lowii,* which must have been seen by Wallace, but not Darwin. This plant attracts small mammals, which sit on top of the pitcher, licking the sweet, sugary secretion on the lid of the plant. Rather than falling into the pitcher, they then defaecate into the pitcher, providing nutrients to the plant (Clarke, Bauer, Lee, Tuen, Rembold & Moran, 2009; Wells, Lakim, Schulz & Ayasse, 2011). What needs to be investigated is whether this is a natural example of the ingestion of sugars causing a gut disturbance, analogous to humans with lactose intolerance or when they eati tri- or tetra-saccharide sugars. It fits Darwin's wonderful legacy that Nature always knows best.

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Figure legends

Fig. 1 Charles Darwin (1809 – 1882)
(a) Aged about 31 soon after his major symptoms began. Charles Darwin in 1840. Watercolour by George Richmond. Down House, Downe, Kent. Darwin Heirlooms Trust. " Charles Darwin " 1840. Chalk and water-colour drawing by George Richmond (1809-96). Reproduced courtesy of the Darwin Heirlooms Trust. English Heritage Photo Library
(b) Aged 50 at the time of the publication of On the Origin of Species.
(c) Aged 70 just 2 years before his death on 19th April, 1882; Frontispiece from (Darwin, 1902)

Fig. 2 The protein domains of the Crohn's risk protein NOD-2
The figure shows the domain structure of nucleotide-binding oligomerisation domain-containing protein 2 (NOD-2) or caspase recruitment domain-containing protein 15 (CARD15).

Fig. 3 Key sugars and substrates cleaved by lactase, and that are digested in the small intestine.
Lactose, glucose, galactose, raffinose, stachyose, phlorizin and a cebroside hydrolysed by the non-lactose site in lactase.

Fig. 4 Disaccharide digestion and absorption in the small intestine
The figure shows the mosaic pattern of lactase expressing cells in the villi of the small intestine, together with the uptake of glucose and galactose from lactose hydrolysis via SGLUT1, which can be inhibited by raffinose or stachyose, and the uptake of fructose into the enterocyte by a different transporter, GLUT5. The monosacchardies are then transported into the blood by GLUT2.

Fig. 5 The Pictet-Spengler reaction
This shows the reaction of methylglyoxal with 5'hydroxytryptamine (5'HT), inactivating the 5'HT.

Table 1 Some proposed causes of Darwin's illness

Proposed diagnosis	Reference
Organic	
Chronic from seasickness	(Anon, 1882a, b)
Poisoning (arsenic, bismuth, calomel – mercury)	(Campbell & Matthews, 2005a; Colp, 1977, 2006; Winslow, 1971)
Heart disease	(Darwin, 1849-1855, 1876)
Nervous indigestion	(Anon, 1882a, b, 1990)
Chronic neurasthenia	(Johnston, 1901)
Chronic eye strain	(Gould, 1903)
Aftermath of fever in Chile	Leonard Huxley, see (Colp, 1977, 2006)
Pyorrhoea	Leonard Huxley, see (Colp, 1977, 2006)
Brucellosis	(Simpson, 1958)
Chagas' disease	(Adler, 1959, 1965, 1989, 1990; Bernstein, 1984; Goldstein, 1989)
General allergy	(Smith, 1992)
Allergy to pigeons	(Gruber & Barrett, 1974)
Acute intermittent porphyria	Dr T.K.With see (King-Hele, 1968)
Diaphragmatic hernia	(Kohn, 1963)
Narcolepsy with diabetes	(Roberts, 1966, 1967, 1990)
Peptic ulcer	See (Colp, 1960, 1977, 1983, 2000, 2006)
Duodenal ulcer	See (Colp, 1960, 1977, 1983, 2000, 2006)
Appendicitis	See (Colp, 1960, 1977, 1983, 2000, 2006)
Smouldering hepatitis	See (Colp, 1960, 1977, 1983, 2000, 2006)
Cholecystitis	See (Colp, 1960, 1977, 1983, 2000, 2006)
Amoeba infection	See (Colp, 1960, 1977, 1983, 2000, 2006)
Ménière's spectrum disorder	(Gordon, 2009a, b)
Systemic lactose intolerance	(Campbell, 2003, 2005a, b; Campbell, Waud & Matthews, 2005; Dixon & Radick, 2009)
MELAS from mitochondrial gene mutation	(Finsterer & Hayman, 2014; Hayman, 2013b)
Gout	(Colp, 2006; Darwin, 1876)
Hyperinsulinism	(Lichfield, 2015)
Malaria	(Hayman, 2009a, 2013a)
Systemic lupus erythematosis	(Hayman, 2009a, 2013a)
Pyroluria	(Hayman, 2009a, 2013a)
Irritable bowel syndrome	(Shanahan, 2012)
Crohn's disease	(Orrego & Quintana, 2007)
Cyclic vomiting syndrome	(Hayman, 2009b, 2013a)
Various skin problems including boils and eczema	(Hayman, 2011; Sauer, 2000)
Psychosomatic	
Reaction to birth trauma	(Hayman, 2013a)
First psychoanalytical theory	(Kemf, 1918)
Hypochondria	(Hubble, 1943)
Psychoneurosis	(Hubble, 1943)
Bereavement syndrome	(Bowlby, 1956, 1984, 1990)
General psychosomatic	See (Colp, 1977, 2006)
General neurosis	See (Colp, 1977, 2006)
Mixed psychosomatic	See (Colp, 1977, 2006)
Psychiatric disease	(Lieb, 2007)
Anxiety state	See (Bowlby, 1956, 1984; Colp, 1977, 2006)
Panic syndrome	See (Bowlby, 1956, 1984; Colp, 1977, 2006)
Nervous indigestion	(Darwin, 1876)
Obsessive compulsive disorder	(Hayman, 2009a, 2013a)
Conflict with religious beliefs	(Hayman, 2009a, 2013a)
Various repressed antagonisms – father, wife, sons	(Hayman, 2009a, 2013a)
Depression	(Alvarez, 1959)

See (Campbell & Matthews, 2005a; Colp, 1977, 2006; Hayman, 2013a, b) for further details.

Table 2 Darwin's correspondence during his time at Ilkley 4th October – 17th December 1859

Letters received

Charles Lyell (4), James Hooker (3), John Murray (1 but several have been lost), Hugh Falconer (1), Charles Kingsley (1), HC Watson (1), TH Huxley (1), Erasmus Darwin (1, weak in the head), Adam Sedgwick (1), Richard Hill (1), Richard Owen (1)

Letters sent

Charles Lyell (11), James Hooker (6), William D Fox 2), William Darwin (2), TH Huxley (6), Louis Agassiz (1), Alphonse de Candolle (1), James Dwight Dana (1), Hugh Falconer (1), Asa Gray (1), John Henslow (1), John Herschel (1), Richard Owen (1), John Phillips (2), Adam Sedgwick (2), Leonard Jenyns (1), Alfred Russel Wallace (1), WB Carpenter (3), John Lubbock (1), Caroline Wedgwood (1), Charles Kingsley (1), Charles Fox (1), TC Eyton (1)

Date from The Correspondence of Charles Darwin Volume , 1859 (Burkhardt & Smith, 1991)

Table 3 The symptoms of lactose intolerance

A. Gut related
- Abdominal pain
- Gut distension
- Flatulence
- Diarrhoea (75%)
- Ileus
- Constipation (25%)
- Nausea and vomiting

B.Systemic
- Headache
- Light headedness and concentration loss
- Tiredness
- Joint pain and/or swelling and stiffness
- Muscle pain
- Allergy – eczema, pruritis, rhinitis, sinusitis, asthma
- Acne
- Heart arrhythmia
- Sore throat and mouth ulcers
- Increased frequency of micturition (urinating)
- Depression

C. Possible
- Hearing loss (3 adults recovered after removing lactose from their diet)
- Infertility (5 became pregnant, after removing lactose from their diet)

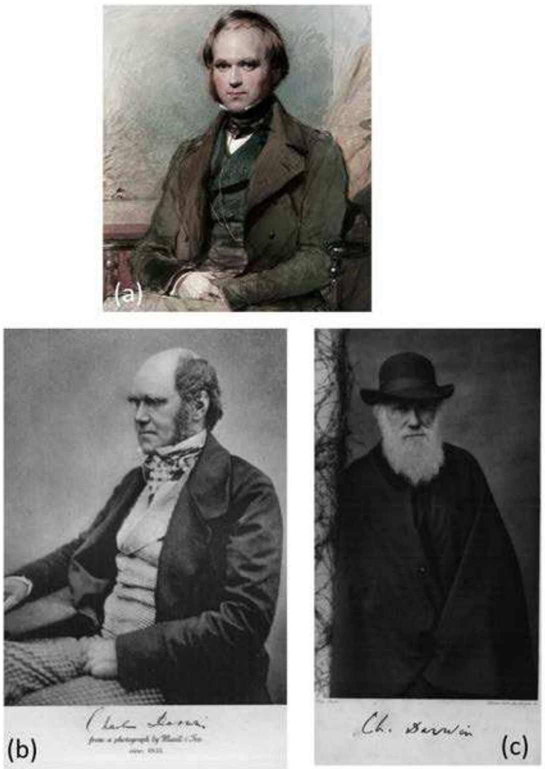
See (Campbell & Matthews, 2005b; Matthews & Campbell, 2000; Matthews, Waud, Roberts & Campbell, 2005; Waud, Matthews & Campbell, 2008) for details

Table 4 Darwin's symptoms versus those of lactose intolerance

Darwin's symptoms as he described them	**Occurrence of Darwin's symptoms**	**Symptoms of lactose intolerance**	**% lactose intolerant patients who have these symptoms**
Nausea and vomiting	Very common	Nausea and vomiting	78
Chronic fatigue and exhaustion	Very common	Tiredness and fatigue	63
Stomach ache and bloating	Common	Gut symptoms (pain, bloating, diarrhoea)	100
Belching (flatulence)	Common	Flatulence	100
Headache	Common	Headache	86
Swimming head, dizziness, memory loss and loss of concentration	Common	Light headedness, memory loss and loss of concentration	82
Rheumatic pain	Common	Muscle and joint pain	71
Skin rash and boils	Common	Allergy (eczema, hay fever, rhinitis, sinusitis, face spots)	40
Mouth and lip sores	Common	Mouth ulcers	30
Palpitations of the chest	Common	Heart palpitations	24
Muscle twitching	Common	Muscle spasms	Occur, but no full data
Dental problems	Common	Weak teeth	Occur, but no full data
Hyperventilation and panic attacks, with sobbing	Common	Panic attacks	Occur, but no full data
Depression	Common	Depression	Common, but no full data

Data from (Burkhardt, Porter, Dean, Topham & Wilmot, 1999; Campbell & Matthews, 2005a; Colp, 1960, 2006; Finsterer & Hayman, 2014; Matthews & Campbell, 2000; Matthews et al., 2005; Waud et al., 2008). Darwin's description of 'belching' may have included what we now call 'flatulence', which the Victorians often found embarrassing to talk about.

Fig. 1 Charles Darwin (1809 – 1882)



(a) Aged about 31 soon after his major symptoms began. Charles Darwin in 1840. Watercolour by George Richmond. Down House, Downe, Kent. Darwin Heirlooms Trust. " Charles Darwin " 1840. Chalk and water-colour drawing by George Richmond (1809-96). Reproduced courtesy of the Darwin Heirlooms Trust. English Heritage Photo Library

(b) Aged 50 at the time of the publication of On the Origin of Species.

(c) Aged 70 just 2 years before his death on 19th April, 1882; Frontispiece from (Darwin, 1902)

Fig. 2 The protein domains of the Crohn's risk protein NOD-2

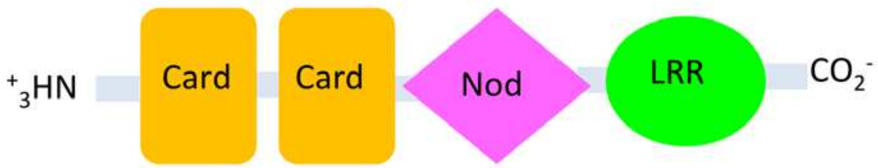


Fig. 2

The figure shows the domain structure of nucleotide-binding oligomerisation domain-containing protein 2 (NOD-2) or caspase recruitment domain-containing protein 15 (CARD15).

Fig. 3 Key sugars and substrates cleaved by lactase, and that are digested in the small intestine

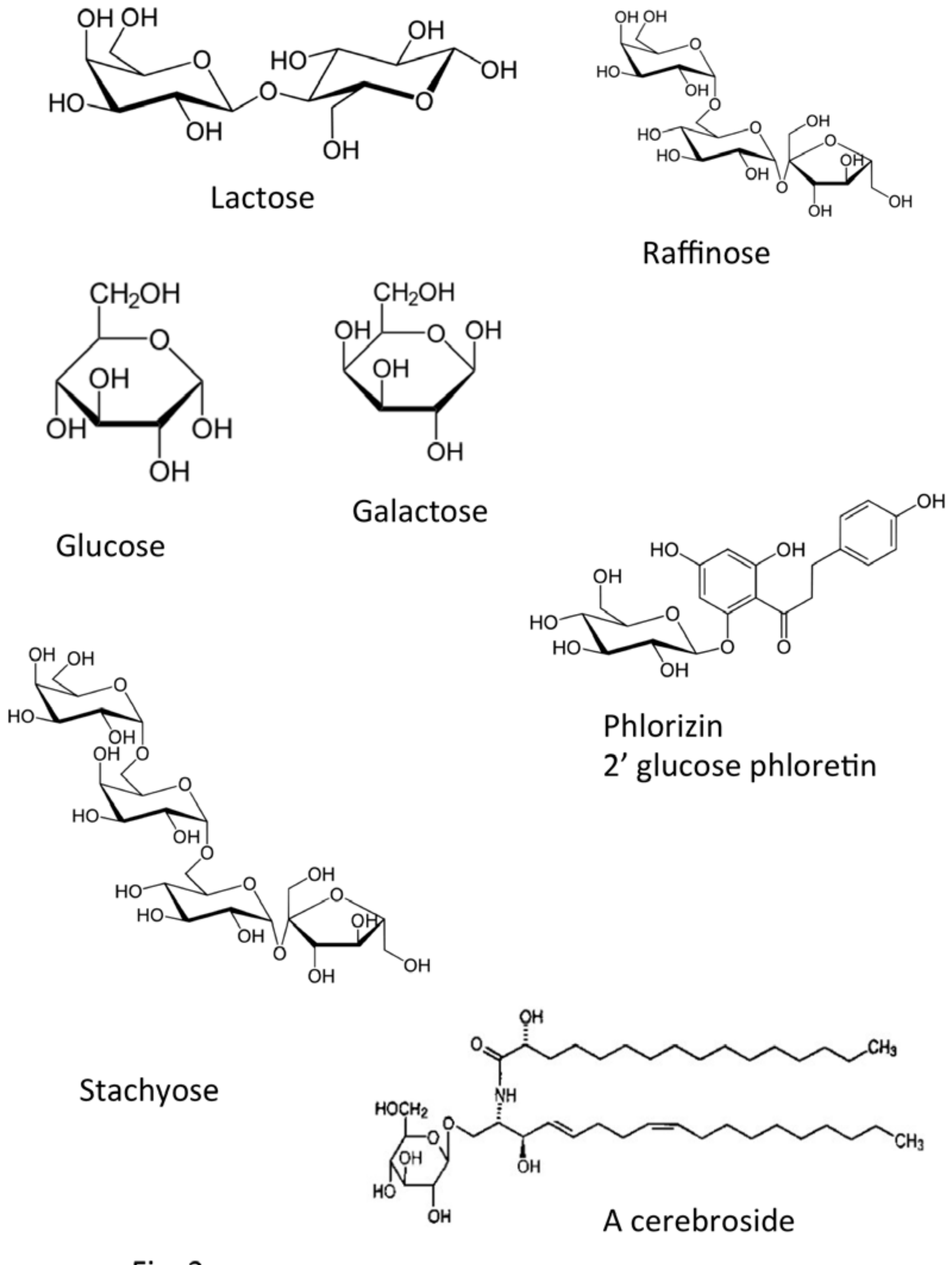
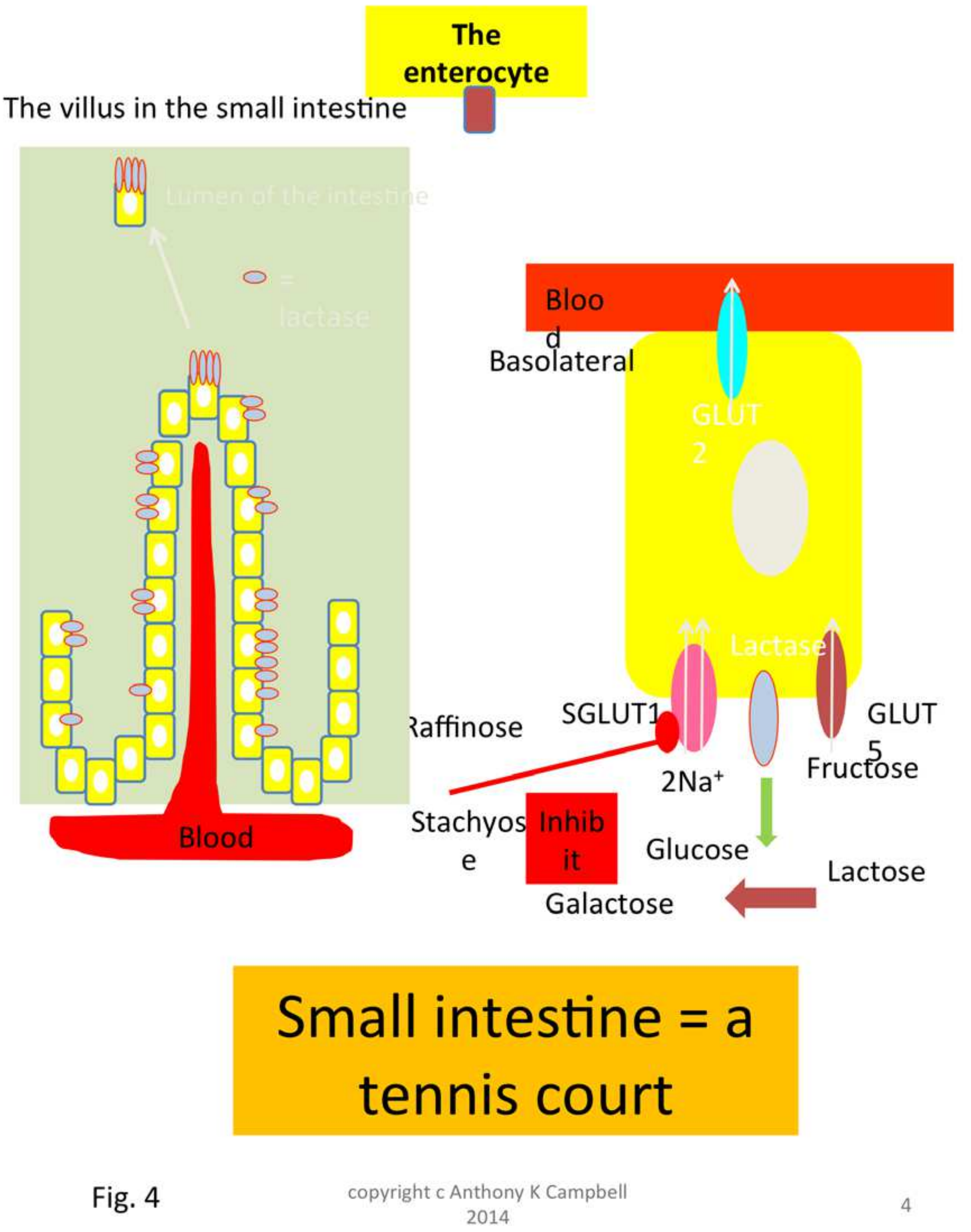


Fig. 3

Lactose, glucose, galactose, raffinose, stachyose, phlorizin and a cebroside hydrolysed by the non-lactose site in lactase.

Fig. 4 Disaccharide digestion and absorption in the small intestine



The figure shows the mosaic pattern of lactase expressing cells in the villi of the small intestine, together with the uptake of glucose and galactose from lactose hydrolysis via SGLUT1, which can be inhibited by raffinose or stachyose, and the uptake of fructose into the enterocyte by a different transporter, GLUT5. The monosacchardies are then transported into the blood by GLUT2.

Fig. 5 The Pictet-Spengler reaction

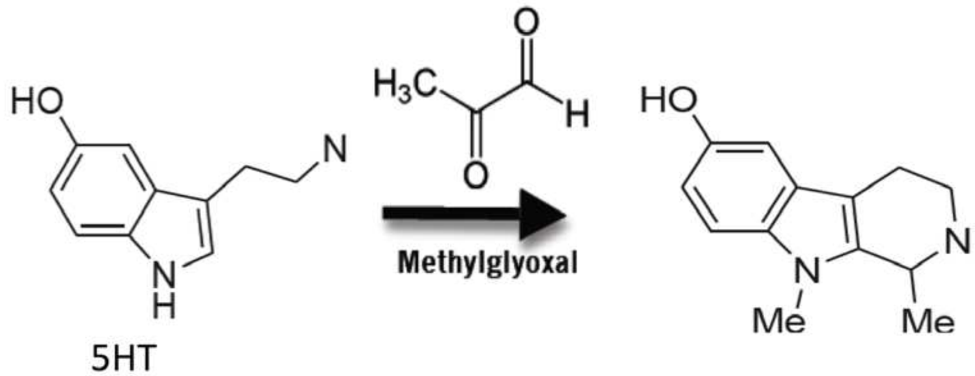


Fig. 5

This shows the reaction of methylglyoxal with 5'hydroxytryptamine (5'HT), inactivating the 5'HT.