CHAPTER NINE

Portrait of a Paradox

Vitamin C and the Many Faces of an Antioxidant

An apple a day keeps the doctor away says the old adage; but is it true? And if it is true, why? The answer to the first question is formulated rather stiffly in our scientific age: a diet containing five 80-gram portions of fruit and vegetables each day reduces the risk of death from heart attacks, stroke and some cancers, especially those of the respiratory and digestive tracts. This is true regardless of our other habits or risks, such as smoking, weight, cholesterol levels and blood pressure. Most people currently eat about three portions a day. Several large epidemiological studies have indicated that increasing average consumption to five portions a day might reduce the risk of cancer by 20 per cent and the risk of heart attacks or strokes by about 15 per cent. Health-conscious individuals really do live longer—it is not just that it feels like it, as Clement Freud once remarked. A 17-year study that examined the mortality of 11 000 people recruited through health-food shops, vegetarian societies and magazines, found their mortality rate was half that of the general population (the study was carried out by doctors at the Radcliffe Infirmary in Oxford and reported in the British Medical Journal, not a partisan health-food magazine). Even allowing for the near-intractable methodological difficulties that blight such studies, and my own reluctance to eat fruit, there can be no question that a diet rich in fruit and vegetables is good for you. The problem is rather how to persuade children and adults, especially in northern Europe and the United States, to modify their diets to include five portions a day—a challenge encapsulated by the Europe Against Cancer slogan 'Take Five!'.

While the benefits of fruit and vegetables are indisputable, the epidemiology of diet, full as it is of bare associations and correlations, is two-dimensional science. For those of an inquisitive cast of mind, the question why is altogether more interesting and complex. Clearly, fruit and vegetables are filled with goodness; yet, perhaps surprisingly, this is about as much as we know for sure. The depth of our ignorance is conveyed pungently in an article by John Gutteridge and Barry Halliwell: "Twenty years of nutrition research have told us that for 'advanced' countries the way to a healthy lifestyle is to eat more plants, a concept familiar to Hippocrates. What it has not told us is exactly why."

If pressed for an explanation, I imagine that most people would say 'vitamin C', 'antioxidants' and suchlike. The reality is of course far more complicated. The health effects of hundreds, if not thousands, of biologically active compounds isolated from fruit and vegetables have been pored over without real consensus. Given the overwhelming detail, it is not surprising that we tend to fall back on a handful of vitamins that people have at least heard of, but which serve really as surrogates for the consumption of many other compounds. A good example
is a study reported by Kay-Tee Khaw and her team at Cambridge in The Lancet in March 2001, which was reported widely and misleadingly by the press: the general projection being that vitamin C lengthens lifespan. In fact, the Cambridge team reported that the risk of death (from any cause) was higher in people with low plasma levels of vitamin C, and conversely, that people with high levels of vitamin C in their plasma were less likely to die within the period studied. The risk of death in people with the highest plasma levels of vitamin C was half that of people with the lowest plasma levels. In the article itself, Khaw and her colleagues were careful to point out that there was no association between vitamin C supplementation and mortality. The association was more generally with dietary intake. And the authors did not discriminate between the amount of vitamin C compared with other factors eaten in the same food at the same time (this is not laziness: asking a specific question often means ignoring superfluous details). No measurements were taken, for example, of plasma levels of vitamin E or beta-carotene. Had they been measured, a similar correlation would almost certainly have been found, for all kinds of antioxidants are abundant in fruit; but that does not mean that they were responsible for the lower number of deaths either. In the Cambridge study, then, plasma vitamin C levels were probably just a surrogate for overall fruit consumption. As to the role of vitamin C itself, we are none the wiser.

Because it is at once so familiar and so inscrutable, we will use the example of vitamin C in this chapter as a springboard to explore the wider function and behaviour of antioxidants. Although often defined simply as a water-soluble antioxidant, vitamin C illustrates many of the difficulties we face in trying to define an antioxidant. Here is Tom Kirkwood, eminent researcher on ageing at the University of Newcastle, and presenter of the 2001 BBC Reith lectures, giving a vivid depiction of the action of vitamin C:

When a molecule of vitamin C encounters a free radical, it becomes oxidised and thereby renders the free radical innocuous. The oxidised vitamin C then gets restored to its non-oxidised state by an enzyme called vitamin C reductase. It is like a boxer who goes into the ring, takes a hit to his jaw, goes to his corner to recover, and then does it all over again.

Kirkwood's description is not wrong, but it is one-sided. His memorable simplicity conceals a can of worms. The molecular action of vitamin C is as simple and repetitive as flipping a coin, yet the effects are varied, unpredictable and utterly dependent on the milieu in which it operates. Just as flipping a coin leads to diametrically opposed outcomes, so too, vitamin C may on the one hand protect against illness and on the other kill tumours, or even people. The food chemist William Porter summed up the conundrum nicely, rising to an anguished eloquence rarely matched in scientific journals: "Of all the paradoxical compounds, vitamin C probably tops the list. It is truly a two-headed Janus, a Dr Jekyll-Mr Hyde, an oxymoron of antioxidants."

Few subjects have polarized medical opinion more violently or senselessly than vitamin C. If one man can be held responsible for this division, it is the great chemist, peace advocate and double Nobel laureate Linus Pauling. We will consider Pauling's life briefly, because his views should not be taken lightly. Neither, we shall see, should
they be taken uncritically.

It is unfortunate, not least for the memory of Pauling himself, that his legacy should be tainted by his controversial views on vitamin C. No researcher had a more profound impact on the advances of chemistry in the twentieth century. One reviewer of his classic 1939 textbook, *The Nature of the Chemical Bond and the Structure of Molecules and Crystals*, went so far as to say that chemistry could now be understood rather than being memorized. Pauling was awarded his first Nobel Prize in 1954 for his "research on the nature of the chemical bond ... and its application to the elucidation of complex substances". In effect, this meant that he had been awarded the prize for the body of his work over the previous twenty years rather than a specific discovery, a move unprecedented in the history of the Nobel Institute. Yet a continuous thread did run through much of Pauling’s early research—an application of the laws of quantum mechanics to the structure of chemical bonds. Pauling set about calculating the length and the angles of individual bonds, using x-ray diffraction, magnetism and measurements of the heat emitted or absorbed in chemical reactions. From the values he obtained, he went on to plot the three-dimensional structure of complex molecules. One of Pauling’s earliest and greatest contributions to chemistry was the idea of *resonance*, in which electron delocalization stabilizes the molecular structure (the electron ‘spreads out’ in space to dilute the charge density). This feature is critical to the action of vitamin C and other antioxidants, as we shall see.

By the mid 1930s, Pauling had begun to apply his analytical methods to the structure of proteins. He demonstrated the importance of minute electrical charges (hydrogen bonds) in stabilizing the three-dimensional shape of proteins, and was the first to describe their grand architectural structures, familiar to all students of biochemistry, such as the alpha-helix and the beta-pleated sheet. By the early 1950s, Pauling was turning his attention to the unsolved structure of DNA. In his famous book *The Double Helix*, James Watson describes the foreboding that he and Francis Crick felt when they heard that the ‘world’s greatest chemist’ was considering the problem of DNA. The pair raced to apply Pauling’s own methods to pip him to the post, and were overjoyed when they realized that, on this occasion, their rival had committed an elementary blunder.

By now Pauling, spurred on by his indefatigable wife Ava Helen, was becoming increasingly committed to anti-war protests. From 1946, through the 1950s and 1960s, Pauling spoke out about the perils of atomic fallout, in particular the risk of birth defects and cancer. In 1957, he drafted a petition to end nuclear weapons testing, and eventually presented the signatures of 11000 scientists to the White House, including those of Albert Einstein, Bertrand Russell and Albert Schweitzer. The petition was widely credited with precipitating the Nuclear Test Ban Treaty, in which the United States and the Soviet Union agreed to cease testing nuclear weapons. Pauling was awarded the Nobel Prize for Peace on 10 October 1963, the same day that the Nuclear Test Ban Treaty went into effect.

Pauling’s anti-war activities inevitably raised the suspicions of the United States government in the early years of the Cold War, when the House Un-American Activities Committee and Senator McCarthy were embroiled in their notorious communist witch-hunt.
During the early 1950s, Pauling had been investigated by the FBI and was refused renewal of his passport, receiving the explanation that "Your anti-communist statements haven't been strong enough". Only in 1954, when he won the Nobel Prize for Chemistry, and the New York Times brought the controversy to light, was he permitted to travel again. Similar struggles plagued his position at the California Institute of Technology. His funding from the National Institutes of Health (NIH) was cut, along with that of 40 other scientists, and he was eventually forced to resign from the faculty in 1963. After an interim of several years at the Center for the Study of Democratic Institutions in Santa Barbara, where he devoted himself to the problems of peace and war, he finally took up a chair in chemistry at Stanford University in 1969. There, he pursued his burgeoning interest in orthomolecular substances, such as vitamin C, which he defined as substances normally present in the human body and required for life. He went on to establish the Linus Pauling Institute for Orthomolecular Medicine, to which he devoted his remaining years.

This brief biography must stand as a measure of the man who, in 1970, published the hugely popular book Vitamin C and the Common Cold, in which he claimed that large doses of vitamin C could prevent or cure the common cold. Pauling and his wife practised what they preached, consuming between 10 and 40 grams of vitamin C each day (several hundred times the recommended daily allowance), even adding spoonfuls to their orange juice. Over the next two decades, Pauling's claims became still more contentious: so-called 'mega-doses' of vitamin C could cure schizophrenia and cardiovascular disease, prevent heart attacks and ward off cancer, perhaps adding decades to our life expectancy. Most controversially of all, Pauling and the distinguished Scottish oncologist Ewan Cameron reported that mega-doses of vitamin C, given intravenously, could quadruple the survival time of patients with advanced cancer, even bringing about complete remission in some cases. The medical profession responded to these claims with suspicion, but the Mayo Clinic in Rochester, Minnesota, did at least conduct three small-scale clinical trials to test the effect of vitamin C in advanced cancer. All three trials failed to detect any benefit. Pauling and Cameron argued that the trials had been designed to fail: in particular, that vitamin C had been withdrawn too soon, and was given orally rather than intravenously. In 1989, the NIH agreed to review 25 case studies, to be selected by Cameron, for plausible evidence that mega-doses of vitamin C might have important effects in cancer. They concluded, in a letter to Pauling in 1991, that the case studies did not provide convincing evidence of a link.

Pauling, then, was a colossus of twentieth-century science, whose work laid the foundations of modern chemistry. He could be insufferably self-assured, but was far from infallible, as Watson and Crick were delighted to discover. His methods were unorthodox. In The Double Helix, written before Pauling’s conversion to orthomolecular medicine, Watson described Pauling’s approach to chemistry as intuitive rather than mathematical; and Pauling referred to his own application of intuitive guesses as the 'stochastic method'. Always the maverick, Pauling felt betrayed by the establishment and was quick to fight his corner, sometimes with barbed personal attacks. His experiences no doubt coloured his attitude to the pharmaceutical industry and the medical
profession, which he termed the ‘sickness industry’, accusing them of misleading the public to bolster drug sales. For their part, doctors dismissed Pauling’s claims for vitamin C as quackery and fraudulence. Journals became reluctant to publish his papers, and the dispute degenerated into a public slanging match. So began a stand-off that continues to this day. Pauling died in 1994, at the age of 93, an embittered man. If he was right, he had solved one of the world’s greatest problems—how to age gracefully—and we would be fools to turn our backs on his simple solution. On the other hand, the woes of old age have hardly been vanquished, even among those who follow Pauling’s example. One might be forgiven for assuming that he must have been mistaken. Was there any truth in his claims?

Vitamin C acquired its status as a vitamin—an essential trace constituent in the diet—for a curious reason. With the exception of higher primates, guinea-pigs and fruit bats, almost all other plants and animals manufacture their own vitamin C. In contrast, we must eat ours, because a common ancestor of the higher primates once lost the gene coding for an enzyme called gulonolactone oxidase, which catalyses the final step in the synthesis of vitamin C. As a result, the entire human race suffers from what amounts to an inborn error of metabolism. Pauling was fond of drawing attention to this deficit in his talks; he would hold up a test tube containing the amount of vitamin C produced by a goat in a single day and say, conciliatory to the end, “I would trust the biochemistry of a goat over the advice of a doctor”.

This apparently strong argument must be flawed. Loss of the gene for gulonolactone oxidase could not have been counterproductive for our primate ancestors, or they would have been eliminated by natural selection. Indeed, the fact that the individuals that lost the gene eventually prevailed among the primates suggests that there may even have been some benefit in its loss. In their authoritative text Free Radicals in Biology and Medicine, Halliwell and Gutteridge suggest one possibility. They note that gulonolactone oxidase produces hydrogen peroxide as a by-product of vitamin C synthesis. This means that high rates of vitamin C synthesis in animals such as the rat could, ironically, impose an oxidative stress. Given an adequate diet of fruit, which is rich in vitamin C, it might indeed be beneficial to consume, rather than synthesize, vitamin C. Of course, this is only true if we do eat enough. One of Pauling’s arguments was based on the observation that gorillas consume nearly 5 grams of vitamin C daily in their normal diet. Our own Palaolithic forebears are thought to have consumed about 400 milligrams daily.

Failure to eat enough vitamin C causes the once-dreaded deficiency disease scurvy. Scurvy is no longer a familiar sight, but once devastated the lives of sailors, who were deprived of fresh foods on long voyages. Scurvy was also endemic among soldiers on military campaigns from the Crusades to the First World War. The disease was a liability for global explorers, who were sometimes at sea for months or years at a time. One outbreak in 1536 afflicted all but 10 of the 110 men wintering aboard the ships of the French explorer Jacques Cartier, founder of Montreal, in the frozen St Lawrence river in Canada. Cartier wrote that “the victims’ weakened limbs became swollen and discoloured whilst their putrid gums bled profusely.” Other symptoms of scurvy include anaemia, spontaneous bruising, fatigue, heart failure and finally...
death. Thirty years after Carder’s winter of sorrows, the Dutch physician Romsen advised sailors to eat oranges to prevent scurvy, and in 1639 the English physician John Woodall recommended lemon juice. With characteristic phlegm, the British Admiralty disregarded this advice, standing firm even after the crew of Lord Anson’s round-the-world expedition of 1740 was cut down by scurvy. Of the 1955 men who set sail, 320 died from fevers and dysentery and 997 from scurvy before Anson returned to England in 1744.

Protesting against the appalling conditions faced by sailors, James Lind, a Scottish naval surgeon, produced a Treatise on Scurvy in 1753, in which he, too, recommended citrus fruits. Unlike his predecessors, however, he had actually proved his theory in the world’s first controlled clinical trial, on board HMS Salisbury in 1747. Lind tested a variety of reputed remedies on 12 members of the crew who had succumbed to scurvy. Two of them received a quart of cider each day; two had oil of vitriol; two received vinegar; two drank sea water; two had oranges and lemons; and the final pair took a medicament prepared from garlic, radish, Peru balsam and myrrh. The two seamen who received oranges and lemons made a speedy recovery and were put to nurse the others. Of the rest, only those receiving cider showed any signs of recovery. Curiously, despite the practicality of his conclusions, Lind did not regard scurvy as a deficiency disease, but rather as the result of a contagion in moist air. He thought of lemon juice as a deterrent that could break down toxic particles.

Lind’s recommendations were acted on by Captain Cook on his two circumnavigations of the globe between 1768 and 1775. Cook had exacting standards, and emphasized the importance of good diet, cleanliness, ventilation and high morale. He supplied his sailors with fresh lemons, limes, oranges, onions, cabbage, sauerkraut and malt. Only one seaman died from scurvy in a total of nearly six years at sea. Even so, the British Admiralty did not capitulate to Lind’s demands until 1795, when it finally agreed to issue lemon juice on British ships. Thanks to the publicizing efforts of Sir Gilbert Blane, physician to the navy, the effect was dramatic. From an average of over 1600 patients with scurvy admitted to the Haslar naval hospital each year, the number dwindled to a mere two between 1806 and 1810. As the late social historian Roy Porter observed wryly, lemons might have done as much as Nelson to defeat Napoleon. The situation did not last long. In a cost-cutting measure typical of the British over the ages, the Admiralty replaced lemons with cheaper limes, which contain barely a quarter as much vitamin C. Scurvy soon reappeared. To add insult to injury, British sailors acquired the nickname ‘limeys’.

The idea that scurvy might be a deficiency disease, rather than an infection, was advanced in the 1840s by George Budd, professor of medicine at Kings College in London, earning him the grand Victorian epithet ‘the prophet Budd’. In a series of articles published in the London Medical Gazette, entitled ‘Disorders Resulting from Defective Nutriment’, Budd prophesied that scurvy was due to the “lack of an essential element, which it is hardly too sanguine to state will be discovered by organic chemistry or the experiments of physiologists in a not too distant future”.

In the event, fulfilment of Budd’s prophesy had to wait another 93 years, in part because the concept of deficiency diseases was set back by Pasteur’s germ theory of disease,
which was then applied with indiscriminate enthusiasm to almost any condition. By the late 1920s, though, a number of researchers were racing to isolate the ‘antiscorbutic factor’ from oranges, lemons, cabbages and adrenal glands. Several workers, notably the Hungarian biochemist Albert Szent-Györgyi, succeeded in isolating white crystals of an acidic sugar, whose properties corresponded to vitamin C, but whose chemical identity remained a mystery. A bit of a joker, Szent-Györgyi proposed the name ‘ignose’, the -ose ending signifying its relationship to sugars, and the ign-prefix his ignorance of its nature. When this name was rejected, he proposed ‘godnose’; and finally, in a single sentence in Nature in 1933, the term ascorbic acid, in reference to its antiscorbutic properties. Progress was fast. The same year, ascorbic acid was synthesized independently by the Polish émigré Tadeus Reich-stein in Switzerland and Sir Walter Haworth in Birmingham, UK, making it not only the first vitamin to be assigned a chemical formula, but also the first to be synthesized by purely chemical means.

Ironically, the concept of vitamin C as the dietary element that prevents scurvy has hampered our understanding of its positive role in the body. The general approach to how much vitamin C we need to eat each day (the recommended daily allowance or RDA) is derived from this negative stance—the prevention of scurvy—rather than any positive criterion. The amount of vitamin C required to prevent clinical scurvy, in other words to hide any obvious signs of disease, is surprisingly small. A series of studies on the inmates of Iowa jails in the 1960s showed that only about 10 milligrams a day are required to abolish the signs and symptoms of scurvy. When the dose is raised to about 60 milligrams a day, we begin to excrete vitamin C in our urine, implying that the excess is superfluous to requirements. The notion that our body pool is saturated by about 60 milligrams a day is supported by the rate of breakdown of vitamin C; the Iowa studies suggested that breakdown products are excreted in the urine at a rate of about 60 milligrams each day. These three factors, then—prevention of scurvy with a margin for error, excretion of vitamin C, and excretion of breakdown products—form the basis of the long-standing RDA of 60 milligrams vitamin C daily.

Although this analysis sounds like a closed case, it is in reality misleadingly simplistic. The case is confounded by both practical and conceptual difficulties. These were scrutinized during the 1990s by Mark Levine, of the NIH. Levine had been a member of the panel convened by the NIH to review Cameron’s 25 case studies in 1989, and has since done more than anyone to bridge the gap between mainstream medicine and the advocates of vitamin C.

Besides querying the accuracy of the early measurements of vitamin C and its breakdown products, which were carried out using insensitive and non-specific tests, Levine questioned the assumptions underlying each of the three factors used to estimate the RDA. First, he said, the amount of vitamin C required to prevent scurvy may be much less than the ideal intake for maintaining bodily functions. We do not know how much less. Second, the threshold of urinary secretion may or may not correspond to the saturation of body pools—it does for some substances and doesn’t for others. For vitamin C, we do not know. Third, the rate of breakdown of vitamin C depends on a variety of factors, including the size of the dose consumed. High doses are broken down faster than low doses, perhaps because the body has less need to
conserve a precious resource. This means that estimates of breakdown based on low doses (such as 30 or 60 milligrams) may be misleading. Thus, Levine stripped away the conceptual basis underpinning the current RDA of vitamin C.

Far from being merely critical, Levine worked up his own recommendations for a rational daily dose of vitamin C, based on the ideal amount required for known reactions, and the saturation of blood levels and other body pools. For the impatient, let me say immediately that he recommends 200 milligrams daily for healthy individuals; that doses above 400 milligrams have no evident value; and that doses of more than 1 gram may not be safe, as they can provoke diarrhoea and induce the growth of kidney stones. Five portions of fruit and vegetables each day corresponds to a daily intake of between 200 and 400 milligrams of vitamin C, so given a sensible diet there is no need for supplementary vitamin C. We shall see that there are other good reasons for not relying on supplemental vitamins. On the other hand, the RDA of 60 milligrams (raised to 90 milligrams in the United States in April 2000) is, according to Levine, too low. To understand his reasoning, and especially the wider ramifications in terms of antioxidant function, we need to look in more detail at what vitamin C actually does in the body.

Quite apart from its antioxidant effects, we need vitamin C for a wide variety of biochemical reactions that help to maintain our normal physiological function. The best known requirement for vitamin C is as a co-factor (a necessary accessory for enzyme function) in collagen synthesis.

Collagen fibres make up about 25 per cent of the total protein content of our bodies, and are familiar to all of us in a melted form as gelatine. In their normal bodily environment, collagen fibres are the most important structural and shock-absorbing component of connective tissues, including bone, teeth, cartilage, ligament, skin and blood vessels. In the absence of vitamin C, collagen fibres do not form properly. Many of the symptoms of scurvy can be attributed to defects in collagen production and maturation. As a result, blood vessels become fragile and wounds heal slowly, if at all. Such vascular degeneration probably accounts for the putrid bleeding gums, swollen joints, spontaneous bruising and, ultimately, as fluids seep out of leaky blood vessels and blood pressure falls, heart failure.

Other symptoms are characteristic of scurvy but not specific to it, including general malaise, fatigue and anaemia. Fatigue affects many millions of people. In some cases, fatigue might be a form of ‘sub-clinical’ scurvy; in other cases it is not. In his Treatise on Scurvy, Lind reported lassitude as an early and invariable symptom. While it is possible that errors in collagen synthesis may contribute to lassitude, such vague symptoms are more likely to relate to the synthesis of a small amino acid called carnitine—which requires vitamin C. We need carnitine to burn fats. When fats are broken down, the component fatty acids must be transported into the mitochondria where they are oxidized to produce energy. The problem is that fatty acids cannot get into the mitochondria by themselves, but must be ferried in attached to carnitine. Carnitine is also responsible for removing left-over organic acids from the mitochondria en route back to the cytoplasm. Without vitamin C, we cannot make enough carnitine to generate energy from fats, and
the mitochondria eventually pollute themselves with toxic organic acids, reducing their ability even to generate energy from glucose. Fatigue might seem a small price to pay.

Vitamin C also has a variety of neuronal and endocrine functions, which are crucial to our physiological and psychological well-being. For example, we need vitamin C to make noradrenaline (norepinephrine), a cousin of adrenaline (epinephrine) which has an important role in modulating our response to stress. We also need it for the correct function of an enzyme called PAM (peptidyl alpha-amidating mono-oxygenase), which is found in many parts of the body, but especially in the pituitary gland. PAM bites the end off a large number of immature peptide hormones and neurotransmitters, and in so doing activates them. Without activation by PAM, the hormones remain inert. If only to downplay the perception of vitamin C as just a water-soluble antioxidant and cofactor in collagen synthesis let me list a few of the peptides that are activated by PAM. They include: corticotrophin-releasing hormone, which stimulates production of steroid hormones; growth hormone-releasing hormone, which promotes growth and influences energy metabolism; calcitonin, which promotes calcium phosphate absorption and distribution in the bones; gastrin, the most powerful stimulant of gastric acid secretion; oxytocin, which stimulates milk ejection and uterine contraction; vasopressin, which regulates water balance and stimulates intestinal contraction; secretin, which stimulates pancreatic and bile secretions; and substance P, a potent vasodilator and sensory neurotransmitter, which mediates our sense of pain, touch and temperature. Given this wide range of actions, the extent to which our normal physiology is fine-tuned by vitamin C is virtually anybody's guess.

Nor is this all. Vitamin C is also taken up by white blood cells. When we are infected with bacteria, white blood cells called neutrophils mount the first defence. In the course of this defence, neutrophils vacuum up vitamin C from their surroundings, using miniature protein pumps in their membranes. The level of vitamin C inside the neutrophils increases tenfold within minutes, and if the infection persists, may reach 30 times the level of resting neutrophils, or 100 times that in plasma, even in someone taking massive oral supplements.

Here, finally, we see an action of vitamin C that seems to be an antioxidant effect, along the lines of that described by Tom Kirkwood at the start of this chapter. Neutrophils need this extra protection to survive the wrath of their own assault, as they turn their immediate environment into a battlefield. The effect is a little like soldiers strapping on their gas masks before releasing chlorine gas onto the enemy. Instead of chlorine gas, neutrophils produce a burst of free radicals and other powerful oxidants (including hypochlorous acid, derived from chlorine), which are responsible for bacterial killing. Vitamin C prevents or delays the demise of neutrophils in a chemical cesspool of their own making, and hastens the death of bacteria, which cannot take up vitamin C or continue to benefit from its presence in their locally demured surroundings. Levine, among others, sees the uptake of vitamin C by neutrophils as a promising avenue for pharmaceutical development in the emerging era of antibiotic resistance.

Finally, what of anaemia? This, too, is a symptom of scurvy, but is not a physiological failure in the sense of
those discussed above. In this case, vitamin C acts on the inorganic iron in food in the stomach and intestines, converting it from the insoluble form usually found in food (Fe$^{3+}$) to the soluble form (Fe$^{2+}$) that we can absorb in our intestines. (This is the reverse of the reaction that took place on a huge scale in the Precambrian oceans, leading to the precipitation of insoluble iron into banded iron formations, discussed in Chapter 2.) Without adequate supplies of vitamin C, we cannot absorb enough iron to stock red blood cells with haemoglobin (which contains iron), and so we develop anaemia.

Such a diverse array of functions lends vitamin C an aura of magic. Yet in each of these cases, vitamin C behaves in exactly the same way at the molecular level, as repetitively as flipping a coin, even if the outcomes are opposed. To see how, let us take a single example in a little more detail. Collagen synthesis illustrates not only how vitamin C works, but also throws light on its antioxidant properties, as well as its more dangerous side.

Collagen can only be manufactured in the presence of molecular oxygen (as we noted in Chapter 4). Oxygen is needed, along with vitamin C, to modify some of its amino acids after they are incorporated into the final protein. The amino acids are hydroxylated, which means that they have an additional hydroxyl (–OH) group attached to them. This hydroxylation enables cross-links to be formed between the individual collagen chains, first to form triple-chain collagen molecules, and then to link these molecules into thicker collagen fibrils. These cross-links are responsible for the tremendous tensile strength of collagen. Without vitamin C and oxygen, collagen cross-links cannot form and the connective tissues are weakened. Not only this, but non-hydroxylated collagen is retained inside the cells that make it, rather than being exported for building purposes. It is also less stable, more sensitive to heat, and more readily broken down by digestive enzymes. Jelly made from scorbutic collagen would be a sorry sight at a tea party.

The mechanism of collagen hydroxylation betrays the secret of vitamin C: it is an electron donor. The oxygen atom in the hydroxyl group comes from molecular oxygen. To attach this oxygen, a single electron must be added to each of the two atoms in molecular oxygen. Because electrons tend to double up as pairs, few compounds can give up a single electron without becoming unstable and reactive themselves. Metals such as iron and copper, which exist in several stable oxidation states, can do so, as can vitamin C. In biological reactions, vitamin C always donates electrons. This and no more. Having said that, it is not profligate with its electrons; under physiological conditions it is most likely to offer them to iron or copper. In the process of collagen synthesis, this is exactly what happens. Vitamin C donates an electron to iron, which is embedded at the core of the enzymes that carry out the hydroxylation reaction. The iron thrusts this electron onto oxygen, which can now be attached to amino acids in collagen. In the process, iron is oxidized to the biologically inactive form (Fe$^{3+}$), until it receives an electron back from vitamin C.

The role of vitamin C in this case is therefore to regenerate the biologically active form of iron by passing an electron to the oxidized form. The hydroxylating enzyme acts as a kind of merry-go-round that uses iron to attach oxygen to amino acids. By providing iron with electrons, vitamin C provides the motive force that keeps the merry-go-round turning.
The triumvirate of iron (or copper), vitamin C and oxygen is at the heart of virtually all the physiological actions of vitamin C. A total of at least eight enzymes use vitamin C as a cofactor. All of these enzymes contain iron or copper at their core. All attack oxygen atoms to amino acids, using the iron or copper. All use vitamin C to regenerate active iron or copper. Essentially the same reaction also accounts for the ability of vitamin C to promote iron absorption in the intestine. In this case, vitamin C donates an electron to oxidized iron, converting it into the soluble form that can be absorbed.

Why is vitamin C used so extensively as an electron donor? There are two main reasons. First, vitamin C is very soluble in water, so it can be concentrated in confined spaces surrounded by membranes (which are made of lipids impermeable to vitamin C). The synthesis of noradrenaline (norepinephrine), for example, takes place in small membrane-bounded spaces, or vesicles, within cells of the cortex of the adrenal glands. The vitamin C concentration inside these vesicles reaches about 100 times that of blood plasma. As vitamin C is consumed by the enzyme dopamine mono-oxygenase, electrons are passed across the vesicle membrane (via an iron-containing protein, cytochrome $b_55$, to regenerate vitamin C within the vesicles. Thus, for periods of days or weeks, the intracellular vitamin C needed for physiological tasks can be ‘insulated’ from changes in plasma levels caused by variations in diet, and maintained at the ideal levels for a particular reaction.

The second reason that vitamin C is used as an electron donor is that the reaction product is fairly stable and unreactive. When vitamin C gives up an electron, it becomes a free radical called the ascorbyl radical. By free-radical standards, the ascorbyl radical is not very reactive. Its structure is stabilized by electron delocalization—the resonance effect first described by Linus Pauling in the late 1920s. This means that vitamin C can block free-radical chain reactions by donating an electron, while the reaction product, the ascorbyl radical, does not perpetuate the chain reaction itself.

Despite its slow reactivity, the ascorbyl radical usually gives up a second electron to produce dehydroascorbate. This molecule is unstable and needs to be caught quickly if it is not to break down spontaneously and irreversibly, and be lost from the body. The continual seeping loss of vitamin C in this way accounts for our need to replenish body pools by daily intake. Even so, we can minimize losses by recycling dehydroascorbate. Several different enzymes bind dehydroascorbate to regenerate vitamin C. These enzymes usually take two electrons from a small peptide called glutathione, and transfer them to dehydroascorbate. Because a pair of electrons are transferred, the regeneration of vitamin C does not produce free-radical intermediates.

The repetitive, coin-flip action of vitamin C is thus to donate single electrons (it donates two of them to form dehydroascorbate). It is regenerated from dehydroascorbate by receiving two electrons from glutathione. This cycle explains not only the enzyme-cofactor effects of vitamin C, but also its antioxidant activity. Even though vitamin C usually gives up electrons to iron or copper, other molecules in search of a single electron can extract one from vitamin C. These include many free radicals, which of course are defined as atoms or molecules with a single unpaired electron (see Chapter 6).

When a free radical reacts, it usually snatches an
Nonetheless, ambiguities aside, the issues raised by even the possibility of pro-oxidant effects give us a perspective on antioxidant ‘networking’ in cells—and also on why Linus Pauling and Ewan Cameron might just have been right about the anti-cancer activity of vitamin C after all.

I should say that there is very little evidence that vitamin C ever acts as a pro-oxidant in people. There are a few indications that the body is aware of the danger, however. In particular, we control our blood plasma levels of vitamin C carefully. Even if we take mega-doses of vitamin C, plasma levels hardly rise. There are two main controls on vitamin C levels in blood plasma—absorption and excretion. The absorption of vitamin C from the intestine falls off drastically the larger the dose. Mega-doses have a laxative effect and cause diarrhoea.

Some advocates of mega-dose vitamin C even recommend ‘titrating to bowel tolerance’—eating nearly enough to cause diarrhoea—to ensure that the maximum possible amount is absorbed. It doesn’t work. Less than 50 per cent of a 1-gram dose is absorbed from the intestine, and most of this is then excreted in the urine. Being freely soluble, vitamin C is filtered by the kidneys, and not all of it is reabsorbed unless an acute shortage leaves the body grasping at straws. Excretion in the urine starts at doses of about 60 to 100 milligrams daily and builds up from there. Nearly all of a 500 milligram dose is excreted in this way. In fact, blood and body pools are saturated by about 400 milligrams of vitamin C each day. It doesn’t matter how much extra you take, you won’t increase your body levels.

While this information is valuable in itself, the fact that the body controls vitamin C levels so tightly suggests that there might be a problem if it did not. Although nobody ever proved that vitamin C is toxic in this way, we should appreciate that neither has anybody proved that vitamin C works as an antioxidant in the body. We know that it can act as an antioxidant, and probably does, but we are far from the kind of rigorous proof that a rocket scientist might demand. Here, for example, is Baltz Frei, current head of the Linus Pauling Institute, writing in 1999: “the current evidence is insufficient to conclude that antioxidant vitamin supplementation materially reduces oxidative damage in humans.” So what about the other side of the coin, actual bodily harm? If the potential toxicity of vitamin C relates to its interactions with iron, then the first place to look for danger is a disease in which iron metabolism is disrupted in some way.

One such condition is iron overload. Rather surprisingly, we have no specific mechanism for removing excess iron from the body, save menstrual bleeding or the shedding of gutlining cells. The rate of absorption of iron therefore needs to be regulated tightly. The inherited form of iron overload, called haemochromatosis, stems from a failure of the normal mechanism for regulating iron absorption in the intestine. Too much iron is absorbed, and in time—40 years or more, depending on diet—this exceeds the body’s capacity to store it safely. Free iron appears in the blood stream. The effect is devastating. Without treatment, the victim can expect to suffer from liver failure (caused by cirrhosis or cancer), weight loss, skin pigmentation, joint inflammation, diabetes and heart failure. Nor is this a rare condition. In western populations, the prevalence is nearly 0.5 per cent, making it one of the most common genetic disorders.

In theory, vitamin C can affect people with iron overload in two different ways. First, it might increase the rate of iron absorption from the intestine. Although there
is no evidence that mega-doses of vitamin C cause iron overload in healthy subjects, it is not at all clear how they affect iron levels in people with haemochromatosis. Second, vitamin C might convert excess iron into the active form that can catalyse free-radical reactions. Whether this might tip the balance between vitamin C acting as an antioxidant or a pro-oxidant in people with haemochromatosis is unknown. Most evidence suggests that it does not, although a few case reports have claimed a detrimental effect. One unfortunate young man in Australia, for example, took high doses of vitamin C for a year before being admitted into hospital with severe heart failure. He died eight days later, diagnosed at autopsy with haemochromatosis. The doctors concluded that the progression of the disease might well have been accelerated by his excessive intake of vitamin C.

But let us consider the risk from another point of view. If there is a dark side of vitamin C, then it might offer an advantage in the treatment of cancer. We know that vitamin C can kill tumour cells grown in the test tube, and that the way in which it does so is dependent on supplies of iron and oxygen. Vitamin C can also kill malarial parasites at certain points in their life cycle, when they are actively accumulating iron from haemoglobin. Is this perhaps an explanation for the Pauling and Cameron findings? It is certainly plausible. The cores of large tumours are often composed of dead or dying cells, which release iron as they degenerate. The fine control of iron metabolism in normal cells may also be lost in cancer cells, which are chaotic and opportunistic in their behaviour. In addition, radiotherapy and chemotherapy cause transient plasma iron overload, which presumably derives partly from the tumour itself. For all these reasons, iron is likely to be present in tumours at a higher concentration than in normal tissues. In the presence of oxygen and vitamin C, then, tumours might conceivably be placed under enough oxidative stress to kill them.

If so, why were Cameron’s results so hard to reproduce? In an incisive reappraisal of the anti-cancer effects of vitamin C in the Canadian Medical Association journal in 2003, Mark Levine and Sebastian Padayatty (also at the NIH) argued that the route of administration holds the key. Pauling and Cameron infused vitamin C intravenously, while the Mayo clinic, in attempting to replicate their data, used high oral doses. When given orally, the low rate of absorption and the high rate of excretion maintain virtually constant plasma levels. Intravenous dosing, on the other hand, side-steps absorption altogether, while the kidneys take some time to remove vitamin C from the blood. For short periods, then, blood levels of vitamin C may reach 50 times their normal saturation point; and this might make all the difference. Padayatty and Levine called for rigorous new trials to test the possibility.

In the meantime, one study of an innovative new cancer therapy does suggest that vitamin C can help kill tumours by exacerbating the effect of free radicals. The treatment is called photodynamic therapy, and was touched on in Chapter 6. The procedure uses light to activate a drug. Once activated, the drug transfers chemical energy onto oxygen, generating singlet oxygen and various free radicals, which attack the tumour. Research teams at the University of Iowa and in China have shown that giving high-dose vitamin C in conjunction with photodynamic therapy enhances the destructive effects of treatment on the tumour. If this turns out to be
an important clinical effect (it is still too early to say), the tarnished reputation of the prophet Pauling may yet be restored.

I have used the example of vitamin C to explore the wider function and behaviour of antioxidants. What have we learnt? The first conclusion is that vitamin C has a repetitive molecular action, constrained by its chemistry. It is not some kind of infinitely flexible superhero, capable of assuming any shape to protect us from evil. All molecular antioxidants are, of necessity, constrained within tight bounds by their chemistry. This does not prevent them from having a wide range of actions. Our second broad conclusion is that a single repetitive action can have many physiological roles besides an antioxidant effect. We have seen that vitamin C is a cofactor for at least eight enzymes, which affect completely different aspects of bodily function, from regular housekeeping tasks, such as collagen synthesis and fat metabolism, to survival measures, such as our response to stress (noradrenaline synthesis) and our perception of pain (activation of substance P). In fact, of all the actions of vitamin C, its antioxidant properties are probably the least well documented. This is equally true of many other reputed antioxidants.

The most compelling evidence that vitamin C does behave as an antioxidant in the body comes from its rapid uptake by neutrophils, where it protects them against their own noxious anti-bacterial effusions. In this respect, it is worth noting that neutrophils only accumulate vitamin C when they are activated by bacteria. Such a sporadic response may reflect no more than the energetic futility of vacuuming up vitamin C when it is not needed, but might equally be a precaution against vitamin C toxicity. This brings us to the third broad conclusion that we can draw from vitamin C. The precise behaviour of an antioxidant depends on its surroundings. Whether vitamin C acts as an antioxidant, or a pro-oxidant, or somewhere in between, depends primarily on its interactions with other molecules. We have seen that vitamin C interacts with some free radicals directly, but also with iron, copper, vitamin E and glutathione. For vitamin C to have a beneficial antioxidant effect, each of these needs to be present in the right amount at the right place, and so each requires its own network of support molecules. If we take a bird’s-eye view, then each of these factors should really be considered antioxidants. Where do we draw the line? To appreciate the difficulty of defining an antioxidant, let us draw this chapter to a close with a quick look at the behaviour of activated neutrophils.

Although neutrophils accumulate vitamin C to 100 times plasma levels, they do not absorb vitamin C itself, but only dehydroascorbate, the oxidized form of vitamin C. Neutrophils have protein pumps in their cell membranes that recognize dehydroascorbate and pump it into the cell. Once inside, the dehydroascorbate is useless until it is regenerated into vitamin C. In neutrophils, this step needs an enzyme called glutaredoxin, which takes electrons from glutathione to regenerate vitamin C. If the whole system is not to grind to a halt, the depleted glutathione needs to be regenerated in turn. Glutathione regeneration is achieved by an enzyme called glutathione reductase, using electrons that would otherwise be used to convert oxygen into water in the course of cellular respiration. This amounts to a long-odds gamble on life itself. The physiological balance of the neutrophil is shifted away from normal respiration—from what amounts to breathing—into an
emergency holding pattern, which is dedicated to regenerating glutathione and thus vitamin C. In other words, activated neutrophils trade taking a breath for protection, in the hope that they will survive long enough to kill the bacteria.

Such heavy betting on the long odds begs the question why vitamin C? As a water-soluble vitamin, it accumulates within the cytosol of the cell. The ‘thin red line’ that holds bacteria at bay is not the inside of the neutrophil, but its surrounding cell membrane, made of fats immiscible with vitamin C. Even when bacteria have been ingested by neutrophils, they are still held in isolation inside the phagocytic vesicles formed from invaginations of the external cell membrane. The neutrophils pour their toxins into these vesicles (as well as the surrounding environment). If they are not to be killed by their own toxins, they must maintain the integrity of their external and internal membranes. If this membrane is damaged in the battle with bacteria, and loses its integrity, the neutrophil will die as surely as we ourselves die when flayed of our skin. In fact, vitamin C is used to rally and, quite literally, revitalize the front-line forces.

Lipid-soluble vitamin E is the foremost defender of the cell membrane. Vitamin E donates electrons directly to free radicals that can damage the membrane’s integrity and so neutralizes them, leaving behind its sacrificial corpse, the a-tocopheryl radical. Vitamin C breathes life back into this near-inert radical, resurrecting it as vitamin E. The reaction takes place without the aid of an enzyme, but its speed depends on the amount of vitamin C relative to vitamin E. The more vitamin C, the quicker the regeneration of vitamin E, hence the stockpiling of vitamin C by neutrophils. At the same time, however, high levels of vitamin C present a danger, especially in the presence of free radicals such as superoxide, which can release iron from proteins (Chapter 6). Vitamin C might change sides and start acting as a pro-oxidant. To stop this from happening, the main agents provocateurs, iron and copper, must be stowed away. Battening down the hatches demands molecular sensors that detect any free iron or copper within the cell, and the capacity to lock away the excess in protein cages (ferritin and caeruloplasmin, respectively). If the storage capacity is limited, new protein cages may need to be manufactured, which in turn requires the transcription and translation of various genes. In all, some 350 genes are expressed by human neutrophils within 2 hours of activation, including the genes for ferritin and caeruloplasmin.

Each link of these entwined chains is critical for the system as a whole to work. The fact that neutrophils protect themselves by accumulating vitamin C, but bacteria do not, hangs by a single thread: bacteria cannot detect dehydroascorbate in their surroundings or pump it into themselves. In neutrophils, the entire response is activated by the presence of dehydroascorbate in the surroundings. The more dehydroascorbate there is, the faster the pumps work. Indeed, neutrophils can be activated in the absence of bacteria by the simple expedient of adding a little dehydroascorbate. Bacteria, on the other hand, remain dormant, even when drowning in pools of dehydroascorbate. They have all the cellular machinery they need to regenerate vitamin C, vitamin E and glutathione, or to hide iron and copper, but are blind to the presence of dehydroascorbate. This single failure may cost them their life. If so, the neutrophils’ gamble on
the long odds will have paid off.

The most striking feature of this scenario is the way in which the whole metabolism of the neutrophil is re-routed in the presence of dehydroascorbate. All of these changes operating together contribute to the overall antioxidant response. We cannot simply define an antioxidant as a molecule with a particular type of action. ‘Seeing’ dehydroascorbate is an antioxidant response. Hiding iron is an antioxidant response. Regenerating glutathione is an antioxidant response. Even lowering the metabolic rate—holding the breath—is an antioxidant response. There is no hard and fast dividing line between factors normally described as antioxidants, such as vitamin C, and physiological adaptations not usually classed as antioxidant responses, such as a reduction in cellular respiration. To have any sense of how such large-scale webs of interactions work in organisms as a whole, we will need to step back from vitamin C. In the next chapter, then, we will take a wider look at how organisms deal with oxidative stress.

1 This quantity (5–10 grams) was based on extrapolations made from measurements in homogenized liver samples, and may bear little resemblance to the amount actually produced by a goat each day.

2 Chronic granulomatous syndrome is an unpleasant condition caused by a mutation in the gene coding for the enzyme NADH oxidase, which generates oxygen free radicals in activated neutrophils. People with the syndrome cannot kill bacteria efficiently and develop chronic suppurating granulomas (abscesses) in the skin, lymph nodes, lung, liver and bones. They also suffer frequently from opportunistic infections. The disease usually develops in childhood and can be treated to some extent with antibiotics.

3 In more technical terms, vitamin C has a relatively neutral reduction potential: it donates electrons to strong oxidants, rather than thrusting them onto weak oxidants, so does not interfere with normal workings of the cell.

4 It is conventional to view the laxative property of vitamin C as a non-harmful side-effect—nothing in comparison with the side-effects of many pharmaceutical drugs—but it may be wiser to view the laxative effect as a deliberate physiological response on the part of the body to potentially toxic levels of vitamin C.

5 Iron overload can also result from the medical treatment of other conditions, notably the thalasaemias, which are caused by an inability to produce haemoglobin at the normal rate. Unless treated with regular blood transfusions, patients with thalassaemia major die in childhood. On the other hand, given regular transfusions, many thalassaemia patients eventually develop iron overload.

6 Advocates of mega-dose vitamin C argue that regeneration of vitamin C by glutathione is a liability: it is slow, drains cellular energy and ultimately deepens the crisis. One rationale for taking mega-dose vitamin C when ill is to side-step this dangerous regeneration step. Such an approach might work outside cells, but cannot work inside cells, where the protection is most urgently needed. The problem is that most cells only recognize the oxidized form of vitamin C, dehydroascorbate. For mega-dose vitamin C to protect cells from within, it must first be oxidized in the blood, then taken up by the cell as dehydroascorbate, and finally regenerated to vitamin C using glutathione. No short cuts here.