Perspectives

The history of vitamin C research in India

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1. Prelude

Vitamin C is perhaps the most controversial, highly publicised yet least understood of all vitamins. Eighty years have passed since its discovery, but till now its precise biological function has remained an enigma. It is perceived by the general public as a panacea, a miracle pill that is capable of curing myriad diseases. Humans, in contrast to many animals, are incapable of synthesising this vitamin and are totally dependent on its dietary intake for their survival. The biosynthetic pathway of vitamin C in animals and its defective counterpart in humans have been completely elucidated, mainly by the pioneering work done in the USA and India. In this essay, besides the discovery of vitamin C by Szent-Györgyi (1928) as well as King and Waugh (1932), I shall elaborate on vitamin C research done exclusively in India.

2. Discovery of vitamin C

The distinctive features of scurvy resulting from vitamin C deficiency were described in the Ebers Papyrus around 1700 BC. They are also found in the texts of Susruta, the great Indian surgeon, around 400 BC; the writings of Hippocrates; and the works of Chang Chi of China ça 200 AD. The knowledge that humans cannot synthesise vitamin C was gained by experience. Between 1500 and 1800 AD, scurvy took a toll of at least two million sailors (McCord 1959). In that period, more seamen died of scurvy than of all other causes combined, including other diseases, shipwrecks, accidents and battle wounds. Scurvy also ravaged whole armies and inhabitants of besieged cities. It was perhaps the main disease resulting from an occupational hazard and, as a nutritional deficiency disease, has caused the most suffering in recorded history (McCord 1959). It was the genius of James Lind (figure 1) that banished this merciless killer of seamen. In 1753, in his book *A treatise of scurvy*, Lind pointed out the importance of lemons, oranges and fresh green vegetables for the prevention and cure of scurvy.

It took another 175 years to discover the antiscorbutic factor vitamin C. The discovery was accidental. In 1927, Albert Szent-Györgyi (figure 2a), a young Hungarian scientist, came to work for one year in the laboratory of F G Hopkins at Cambridge, England, with a fellowship from

Figure 1. The Scottish doctor James Lind with "*A Treatise of the Scurvy*" (published in 1753) in his hand. Lind discovered the importance of lemons, oranges and fresh green vegetables for the prevention and cure for scurvy.

Keywords. Biological function; biosynthesis; emphysema; genetic defects in human; vitamin C

the Rockefeller Foundation. Szent-Györgyi's field of interest was oxidation reactions of compounds present in both plant and animal tissues.

In Hopkins's laboratory, his objective was to isolate some redox substances present in ox adrenal glands. He measured the amount of reducing factor in samples by their power to decolourise iodine. While extracting and concentrating redox compounds from ox adrenal gland, he isolated some sugar-like crystals that he knew nothing about. This ignorance was apparent from the title of the paper he submitted to *Biochemical Journal*: "Observation on the function of peroxidase systems and the chemistry of the adrenal cortex: description of a new carbohydrate derivative" (Szent-Györgyi 1928). He was so ignorant of the nature of the carbohydrate derivative that he first named it ignose (ign for ignorance and ose for sugar). However, the Editor of *Biochemical Journal* did not accept the name. Later, Szent-Györgyi named it godnose (God only knows). However, the Editor objected to this name as well. Finally, the structure of the carbohydrate was elucidated by the famous English chemist W M Haworth at the University of Birmingham and was named hexuronic acid (hex = six carbon uronic acid) (figure 3). During the same period, King and Waugh (1932) of Columbia University isolated the antiscorbutic factor from lemon juice and named it vitamin C. It had all the characteristics of Szent-Györgyi's hexuronic acid.

In January 1931, Szent-Györgyi left Cambridge and returned to Hungary to chair the medical chemistry department at the University of Szeged. In the fall of 1931, an American post-doctoral fellow, Joseph Svirbely, joined Szent-Györgyi's research team. Svirbely had been working with C G King (figure 2b) at the University of Pittsburgh, trying to isolate vitamin C. Szent-Györgyi gave him the remains of the hexuronic acid he had isolated at the Mayo Clinic and asked him to test it on guinea pigs with induced scurvy. Repeated trials proved that hexuronic acid was, in fact, vitamin C. Szent-Györgyi had suspected this, but had put the project aside rather than take up the messy, expensive and labour-intensive animal studies required. King, meanwhile, had also been close to reaching a similar conclusion. Svirbely wrote to his former mentor in March 1932, telling King what he had found at the Szeged laboratory, adding that he and Szent-Györgyi were submitting a report to *Nature*. On 1 April 1932, *Science* published King's paper entitled "The chemical nature of vitamin C" claiming that he had discovered vitamin C, which was identical to hexuronic acid. King cited Szent-Györgyi's earlier work on hexuronic acid but gave him no credit for the discovery of vitamin C. The story of the discovery was quickly picked up by the American press. Two weeks later, astonished and dismayed, Szent-Györgyi and Svirbely sent off their own report to *Nature* entitled "Hexuronic acid as the antiscorbutic factor" (Svirbely and Szent-Györgyi 1932), challenging King's claim to the discovery. A bitter controversy ensued. King, as was well-known, had been working on the problem for over five years; he had many supporters who were ready to vilify Szent-Györgyi as a plagiarist. Yet European and British scientists also knew of Szent-Györgyi's long research on this antioxidant substance and accepted his claim. Ultimately, Szent-Györgyi's claim prevailed. In October 1937, he was awarded the Nobel Prize for Physiology or Medicine "for his discoveries in connection with the biological combustion processes, with especial reference to vitamin C and the catalysis of fumaric acid".

In fact, Szent-Györgyi was surprised when informed of the Nobel Prize. Here was a scientist who worked only for a year on a problem with quite a different objective and without doing a single animal experiment but got full credit for discovering vitamin C (Carpenter 1988). C G King, who

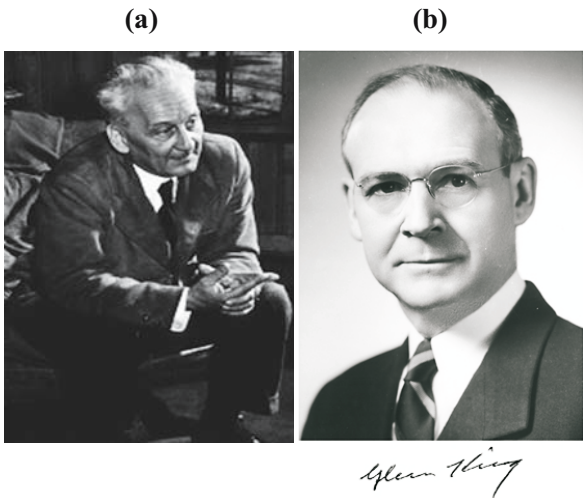

Figure 2. **(a)** Albert Szent-Györgyi (1893–1986). Photo by J W McGuire. Szent-Györgyi discovered hexuronic acid from the adrenal gland in 1928. **(b)** Charles Glen King (1896–1988). King discovered vitamin C from lemon juice in 1932 and showed that it had all the characteristics of hexuronic acid.

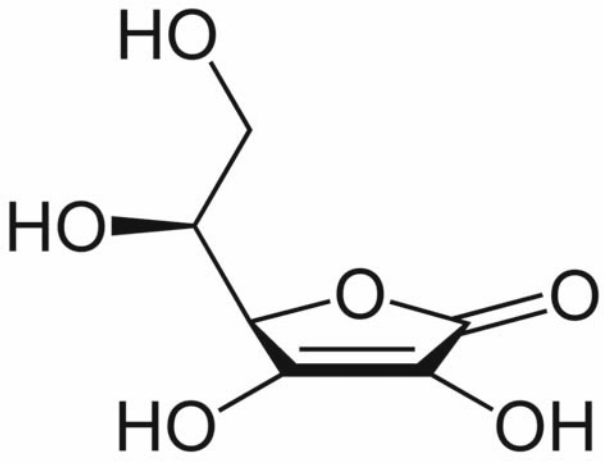

Figure 3. Structure of hexuronic acid (vitamin C).

had published five papers and isolated vitamin C, did not. In the words of Szent-Györgyi himself: "I was not acquainted with animal tests in this field and the whole problem was, for me too glamorous, and vitamins were, to my mind, theoretically uninteresting. Vitamin means that one has to eat it. What one has to eat is the first concern of the chef, not the scientist" (Szent-Györgyi 1963).

3. Initiation of vitamin C research in India

B C Guha (figure 4), one of the founders of modern biochemistry in India (Chatterjee and Burma 2004), pioneered vitamin C research in India. Between 1927 and 1928, while Guha did research in the laboratory of F G Hopkins at Cambridge, he was in close association with Szent-Györgyi. In fact, Guha's interest in vitamin C originated at Cambridge during his stay in 1928. After the discovery of vitamin C, the knowledge of the cause of and cure of scurvy was clear. However, the following questions remained unsolved: (i) How is vitamin C synthesised by most animals? (ii) Why are humans incapable of producing the vitamin? (iii) What is the precise biological function of vitamin C? After his return from Cambridge, Guha joined the Bengal Chemical and Pharmaceutical Works (BCPW) at Calcutta (now Kolkata). The BCPW had been established by P C Ray, the doyen of Indian chemists. There, he founded a laboratory for nutritional research and started work immediately. Guha believed that research should be carried out to alleviate human suffering and not a single day should be lost. During his short stay in BCPW (1932–1935), Guha published 38 papers, most of which were on nutritional science with special reference to vitamin C. None of the above-mentioned questions were addressed at that time. In 1936, at the age of 32, Guha joined the Department of Applied Chemistry of Calcutta University as the Rashbehari Ghosh Professor and Head of the Department. He started a full-fledged school of nutrition, but in course of time he became involved with various administrative jobs. Although Guha continued research on vitamin C through his students, the important questions in the field remained unaddressed.

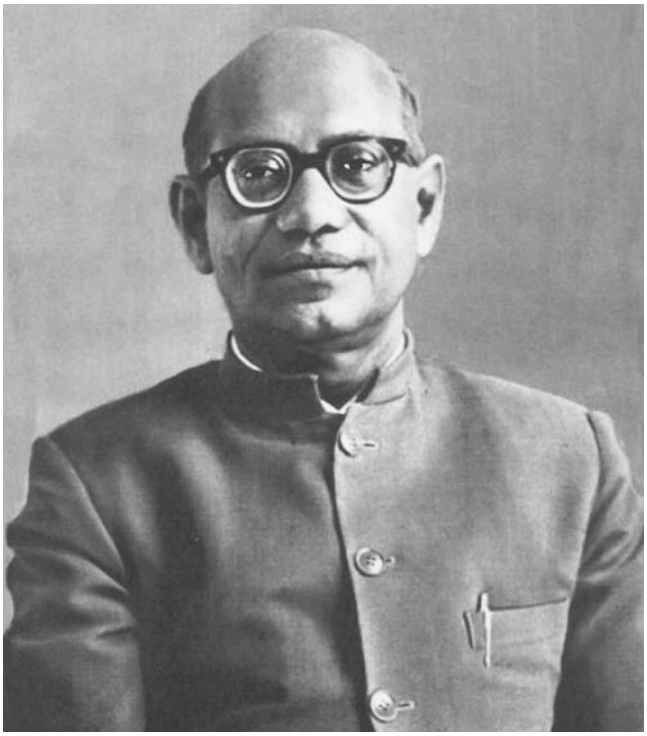

Figure 4. B C Guha (1904–1962).

4. How I came to vitamin C research

After an MSc in Applied Chemistry at Calcutta University in 1952 with a specialisation in oil technology, I started research for a PhD in the same department on the reclamation of rancid fat. Guha rejoined the Department of Applied Chemistry in November 1953. By that time, although a significant portion of my PhD research had already been done, I approached Guha, whom I considered an ideal mentor, and was inducted into his vitamin C research group. Before joining Guha's laboratory, I had limited knowledge of biochemistry and nutritional science, not to speak of vitamin C. About this vitamin, I knew only what Szent-Györgyi used to think: "A vitamin is a substance that makes you ill if you do not eat it."

I had the perception that research means four things: "Brains with which to think, eyes with which to see, machines with which to measure and the fourth, money." In Guha's laboratory, there was no dearth of money and machines. So, I concentrated on seeing what my fellow researchers would see and used my brains to think of what everyone had seen but nobody had thought of. It took me quite some time to adapt to the new intellectual environment. The prime difficulty was to discover a suitable technique for studying the biosynthesis of vitamin C *in vitro*. The classical method for determining the ability of an animal to synthesise vitamin C was to feed the animal a scorbutogenic diet for a prolonged period. Obviously, the method was laborious and time consuming. After much trial and error over three years, I discovered an *in vitro* system by which I could determine whether a tissue from an animal was capable or not of synthesising vitamin C (Chatterjee *et al.* 1957).

5. The pathway of vitamin C biosynthesis in animals

C G King and his co-workers in the USA had shown that in the rat vitamin C is synthesised from glucose via the glucuronic pathway of metabolism:

D-glucose → D-glucuronic acid → L-gulonic acid → L-gulonolactone → L-ascorbic acid (vitamin C)

(Horowitz and King 1953a,b; Kar *et al.* 1962) (figure 5). But how L-gulonic acid is converted to L-gulonolactone and thence to ascorbic acid remained unsolved. We discovered and characterised two enzymes, namely, aldonolactonase (EC 3.1.1.18, L-gulonolactone hydrolase, which reversibly converts L-gulonic acid to L-gulonolactone) and L-gulonolactone oxidase (LGO, EC 1.1.3.8, L-gulonolactone: oxygen 2-oxidoreductase, which oxidises L-gulonolactone to 2,keto-L-gulonolactone) (Chatterjee *et al.* 1958, 1959a,b, 1960a,b; Kar *et al.* 1962.). 2,Keto-L-gulonolactone is spontaneously converted to L-ascorbic acid. No enzyme is needed for this step.

$$\text{L-gulonolactone} \xrightarrow{LGO} \text{2,keto-L-gulonolactone} \xrightarrow{spontaneous} \text{L-ascorbic acid.}$$

2,Keto-L-gulonolactone is an extremely unstable intermediate and here I should mention how a trivial observation can make a significant contribution. In the presence of L-gulonolactone oxidase, L-gulonolactone consumes one mole of oxygen to produce one mole of ascorbic acid. In determining the effect of pH on this reaction, I observed that at pH 9.0 the oxygen consumption remained the same, but there was no production of vitamin C. This led me to think that a precursor of ascorbic acid might be trapped in the incubation medium. This was actually the case, and the trapped intermediate was identified to be 2,keto-L-gulonic acid which, on acidification, was lactonised and spontaneously converted to L-ascorbic acid (Chatterjee *et al.* 1959a,b). The observation was very simple, but it took many failures over one year to establish this simple fact.

At this point, I shall digress a little and mention an important event in my research career. The mid-fifties to the early sixties was an active time for vitamin C research. Many famous scientists ventured into this field. Between 1956 and 1958, the famous biochemist Albert Lehninger reported that in animals the pathway of

$$\text{ascorbic acid biosynthesis was L-gulonate} \xrightarrow{Dehydrogenase} \text{3,keto-L-gulonate} \xrightarrow{Lactonase} \text{3,keto-L-gulonolactone} \rightarrow \text{L-ascorbic acid}$$

(Hassan and Lehninger 1956; Grollman and Lehninger 1957; Bublitz *et al.* 1958). These results were at variance with what we had reported earlier. So, one of us was wrong. Despite limited research experience and little knowledge of biochemistry, I had confidence in my findings. I expressed my view to the senior members of my laboratory that, with all due respect to Lehninger, he might be wrong. They ridiculed me. How dare I think that Lehninger, a top biochemist of the world, was wrong and I was right? I repeated the work of Lehninger with meticulous care and finally discovered that the methodology that was used by Lehninger to measure vitamin C was incorrect. I wrote two papers pointing out these lacunae and sent it to *Biochemical Journal* and they were accepted for publication (Chatterjee *et al.* 1960a, b). To my surprise, before my papers were printed, there was a preliminary note published by Bublitz and Lehninger in *Biochim. Biophys. Acta* (1959) in which the authors wrote: " …..the ascorbate formed from L-gulonate by the action of DPN-L-gulonate dehydrogenase was not identical with authentic ascorbic acid….". I had been vindicated. My work on vitamin C was referenced in Lehninger's first edition of his now indispensable textbook of biochemistry (Lehninger 1978). As a young scientist

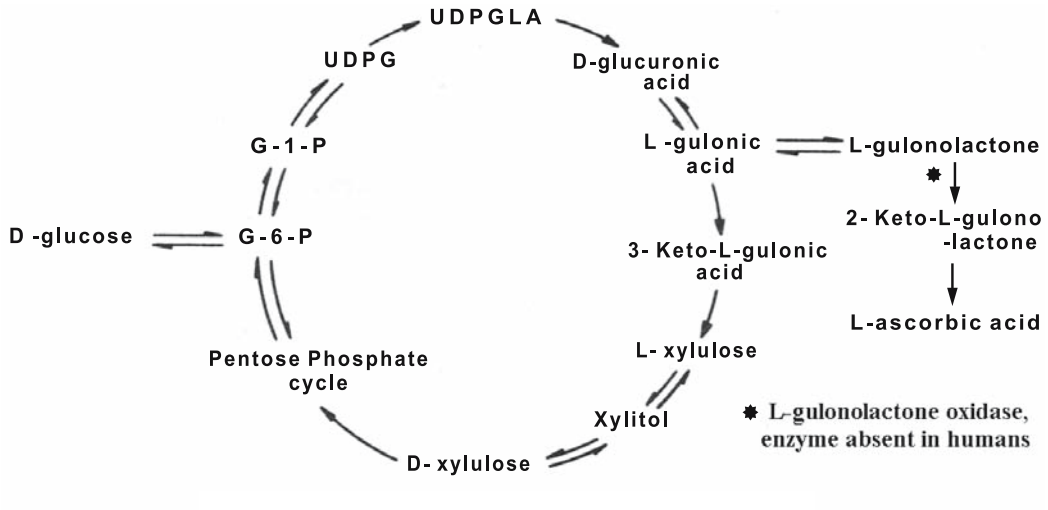

Figure 5. Pathway of vitamin C synthesis in animals, showing the key terminal enzyme L-gulonolactone oxidase, a lack of which causes scurvy in humans (from Chatterjee IB 1978 *Wld. Rev. Nutr. Diet* **30** 69–80).

in 1960, I was further encouraged when I saw that our observations on vitamin C became the basis of an editorial written by F G Young, indicating a correlation between the defect of a single gene (L-gulonolactone oxidase) and the development of molecular pathology (Young 1960). I was also gratified by the comment made on our work (Chatterjee *et al.* 1961) by C G King, the discoverer of vitamin C: "This discovery adds a new and very interesting landmark to the history of vitamin C" (King 1961).

After being awarded the degree of Doctor of Science of Calcutta University in 1961. I proceeded for postdoctoral studies in the Department of Physiological Chemistry, University of California at Los Angeles (UCLA). In September 1961, I joined the laboratory of Ralph W McKee, a co-worker of Henrik Dam, who won the Nobel Prize for the discovery of vitamin K. On 20 March 1962, the heartbreaking message reached me of the sudden and untimely death of B C Guha at the age of 57 years. To me it was a bolt from the blue. As a mentor, Guha had induced in me the essence of research imbibed at London and Cambridge under Sir Jack Drummond and Sir Frederick Gowland Hopkins.

In UCLA I had another learning experience about confidence in research. McKee had given me a problem on the immunological aspect of Ehrlich ascites tumour. At that time, cancer research was in its early infancy and Ehrilich ascites cells occupied a major part of cancer research. McKee's observation was that if irradiated ascites cells were injected into normal C7 black mice, the mice became immune. Injection of viable ascites cells into the immune mice did not produce any tumour. The hypothesis was that injection of irradiated cells produced tumour-specific antibodies. After working for six months I came to the conclusion that injection of irradiated cells produced only increased levels of γ-globulin and not tumour-specific antibody. When I reported these findings to McKee, I found that he was reluctant to accept my inference, particularly because I had no experience in immunology. However, I was finally able to convince him of the veracity of my findings. McKee was a large-hearted man and thereafter gave me the green signal to continue my research on vitamin C, my primary interest. From then on, vitamin C has been with me all along. While at UCLA, I published three papers on vitamin C (e.g. Chatterjee *et al.* 1965).

6. Evolution and the biosynthesis of vitamin C

After returning from the USA in 1964, I joined the Department of Biochemistry of Calcutta University as a lecturer and continued my research on vitamin C, addressing the question of the evolutionary loss of vitamin C-synthesising capacity in humans. The ability to synthesize ascorbic acid is absent in insects, invertebrates and fish. Biosynthetic capacity evolved in amphibians and was tissue specific. Biosynthesis occurred first in the kidney of amphibians, resided in the kidney of reptiles, was transferred to the liver of mammals, and finally disappeared from guinea pig, bats, monkeys, apes and humans (Chatterjee 1973). Similar observations were made in birds (Chaudhury and Chatterjee 1969). Primitive birds synthesise vitamin C in the kidney. The more evolved Passeriformes produce it in the liver, while a number of more evolved passerines are incapable of synthesising the vitamin (figure 6).

The inability of guinea pig, bats, monkeys, apes and humans as well as the highly evolved passerine birds to synthesise the vitamin is due to a common defect – the absence of the terminal enzyme L-gulonolactone oxidase (Chatterjee *et al.* 1960a,b, 1961, 1975; Chatterjee 1978). Amphibians were the first to develop the capacity to synthesise vitamin C about 330–340 million years ago and the gene mutation leading to loss of the capacity took place in the common ancestor of humans and other primates about 25 million years ago. Loss of the gene in guinea pig and humans was probably due to a deletion mutation ((Nishikimi *et al*. 1994). The mutants did not become extinct because the environment furnished vitamin C and the species continued to survive (Nandi *et al.* 1997). On the basis of our observations, the famous nutritionist Nevin S Scrimshaw

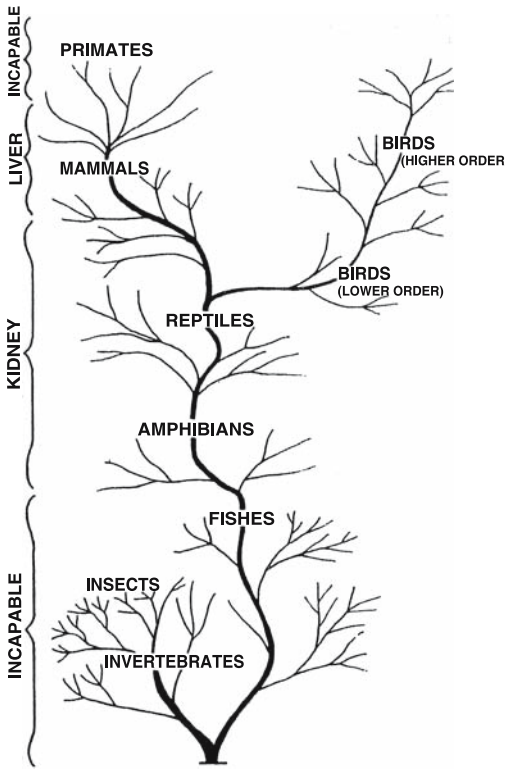

Figure 6. Vitamin C-synthesising ability of different species of animals in a schematic phylogenetic tree (Chatterjee 1973).

wrote an article in *Scientific American* (Scrimshaw and Young 1976). He commented that the inability of humans to synthesise vitamin C is not only of nutritional significance and part of their biological evolution, but also has shaped social evolution. It has been suggested that the migration of human groups to the northern regions of the earth was slowed by the limited amounts of ascorbic acid in the foods available in those areas during the long winter months (Scrimshaw and Young 1976).

7. Biological function of vitamin C

After answering two questions: (i) how is vitamin C synthesised by most animals and (ii) why are humans incapable of producing vitamin C, I started on work to answer the third question: what is the precise biological function of vitamin C? Eight decades have passed since the discovery of vitamin C, but its biological function has remained a mystery. The biological function of vitamin C is probably related to the evolutionary significance of the biosynthesis of vitamin C in terrestrial vertebrates. The evolution of amphibians from fish took place in the Devonian, when the oxygen concentration in water was about 0.5% but the atmospheric oxygen concentration was 15–18% (Graham *et al.* 1995). Thus, during evolution from an aquatic medium to the terrestrial regimen, newly evolved vertebrates were exposed to an environmental oxygen concentration 30–36 times that of their aquatic ancestors. Such a high concentration of extremely toxic oxygen acted as an acute constraint for respiratory adaptation and survival on land. Apparently expression of the L-gulonolactone oxidase (LGO) gene took place in early tetrapods under selection pressure to provide the newly evolved terrestrial vertebrates with adequate amounts of vitamin C to protect their tissues against oxygen toxicity (Nandi *et al*. 1997). This hypothesis supports the numerous reports about the well-established antioxidant function of vitamin C. However, some reports indicate that LGO activity is present in partly terrestrial lung fish (Chatterjee 2008). This would suggest that the ancestral fish LGO gene might have been passed on to amphibians about 400 million years ago.

The concept that megadoses of vitamin C could have a beneficial effect was the brainchild of Linus Pauling, winner of two Nobel prizes (Chemistry Prize in 1954, Peace Prize in 1962) and a genius of the twentieth century. Based on some calculations such as (i) the capacity for biosynthesis of vitamin C by rats and (ii) vitamin C intake through various plant materials ingested by apes, Pauling suggested that the intake of vitamin C should be about 2.3 g a day for the maintenance of general health, and increased intake up to 10 g/day for combating infectious diseases, including the common cold as well as prevention and cure of cardiovascular diseases and cancer (Pauling 1970). Before Pauling stepped into the field, research on vitamin C was based on the concept that the sole function of vitamin C was to prevent scurvy. The knowledge that vitamin C was needed for the prevention and cure of scurvy was amply satisfying for many scientists, particularly those in the medical profession. The prevalent notion was that the disease and its cure had been explained and therefore no longer needed investigation. But that was not accepted by either Pauling or Szent-Györgyi. In reply to a letter from Pauling, Szent-Györgyi wrote "As to ascorbic acid, right from the beginning I felt that the medical profession misled the public. If you do not take ascorbic acid with your food you get scurvy, so the medical profession said that if you do not get scurvy you are all right. I think that this is a very grave error. For full health you need much more. I am taking, myself, about 1 g a day. What I can tell you is that one can take any amount of ascorbic acid without the least danger" (Szent-Györgyi 1970). In fact, Pauling's concept of the health benefits of vitamin C, particularly the benefit of megadoses of vitamin C, has been the subject of debate and controversy. He was severely criticised by popular writings in the *Medical Letter* (25 December 1970), *New York Times* (3 January 1971), *Consumer Report* (February 1971) and *Reader's Digest* (1971). The impact of these criticisms was so great that Pauling was debarred from communicating research papers of other scientists to the *Proceedings of the National Academy of Sciences* (PNAS). In 1992 Pauling regretfully wrote to me, "I must tell you that the Board of National Academy of Sciences has made a ruling that I am not permitted to submit papers for publications in the *Proceedings*, except those of which I myself am one of the authors."

The amount of vitamin C needed to prevent scurvy in humans is only about 10 mg/day. However, despite epidemiological and some experimental studies, it has not been possible to show conclusively that higher than an antiscorbutic intake of vitamin C has clinical benefit (Padayatty *et al.* 2003). The pertinent point is how would one determine criteria to demonstrate the health benefit of vitamin C? In fact, I also did not observe any extra beneficial effect of megadoses of vitamin C in the maintenance of normal health in guinea pigs (Chatterjee 1973). On the other hand, megadoses of vitamin C appear to have beneficial effects on the outcome of other medical conditions. Recently, using a guinea pig model, we have demonstrated that moderately large doses of vitamin C prevent cigarette smoke-induced emphysema. It should be mentioned that cigarette smoking is by far the commonest cause of emphysema, accounting for about 95% of cases (Barnes *et al.* 2003).

8. Research on cigarette smoke and vitamin C

I consider that the prime biological function of vitamin C is associated with its redox property, which explains

its antioxidant effect. In the mid-nineties we performed some experiments to study the antioxidant effects of vitamin C. We developed an *in vitro* system where addition of nicotinamide adenine dinucleotide phosphate (NADPH) to microsomal suspensions of tissue (lung, liver, heart and kidney) led to oxidative damage accompanied by proteolysis of the microsomal proteins. This damage was exclusively prevented by vitamin C (Mukhopadhyay and Chatterjee 1994a, b). One evening, while I was walking down the corridor of the laboratory, I saw somebody smoking in the distance and observed that the smoke was curling up in the air. I knew that cigarette smoke contains several oxidants. At once, I thought of an important question: would the oxidative damage of proteins induced by cigarette smoke be prevented by vitamin C? The very next morning we performed an experiment to answer this question. We added a few microlitres of aqueous extract of cigarette smoke to guinea pig lung microsomal suspension and, to our surprise, we observed that cigarette smoke solution did cause oxidative damage and proteolytic degradation of the microsomal proteins, which was almost completely prevented by vitamin C (Ghosh *et al.* 1996; Panda *et al.* 1999, 2000). Since cigarette smoke-induced lung damage is apparently caused by destruction of the alveolar and septal cells, we addressed two more questions: (i) would cigarette smoke-induced oxidative damage of lung proteins lead to pulmonary emphysema that could be prevented by vitamin C, and (ii) what component of cigarette smoke was responsible for protein damage in the lung? Eventually, we solved both problems. This was possible only because I was completely ignorant about the nature and composition of cigarette smoke. If I knew then that cigarette smoke contains about 4000 compounds, I would never have dared to launch research on it.

In 1995, when I formally retired from the post of Professor in the Department of Biochemistry at Calcutta University, R N Basu, the then Vice-Chancellor of Calcutta University, requested me to continue my research in the newly established Dr B C Guha Centre for Genetic Engineering and Biotechnology of the University of Calcutta (where I had been the founding coordinator). After five years, we finally isolated from cigarette smoke a long-lived semiquinone radical and characterised it as p-benzosemiquinone (p-BSQ) (Banerjee *et al.* 2008). Earlier studies on the electron spin resonance of cigarette smoke condensate had indicated that semiquinones are present in cigarette smoke, but these had not been isolated and characterised separately (Pryor *et al.* 1998). An orthodox classical chemist would be skeptical as to how a semiquinone radical could be isolated. That is why it took a long time to get the paper published. The paper was accepted after several rejections. In spite of all the required experimental data, one reviewer believed that p-BSQ could not be isolated and purified. We could do this because p-BSQ is a long-lived radical, the half-life of which in the solid state is about 48 h. We examined about 15 brands of cigarettes, both Indian and international. All the brands contain p-BSQ. At this point, I should express my gratitude to Dr R A Mashelkar, the then Director General of the Centre for Scientific and Industrial Research (CSIR). In 2001, during a leisurely conversation at the Bose Institute, Kolkata, I told Dr Mashelkar that we were probably on the verge of isolating a hazardous compound from cigarette smoke, which would explain the cause of lung damage due to smoking. Dr Mashelkar was very excited and told me that we should not publish the result; CSIR would patent this finding and bear all costs. Later, we observed that in a guinea pig model, p-BSQ very nearly mimics the protein damage, apoptosis and pulmonary emphysema caused by cigarette smoke (Banerjee *et al*. 2008). The process of isolation of p-BSQ from cigarette smoke has been patented (US Patent 2005) and is being patented in different countries of the world. At the request of CSIR, I had to reluctantly do another piece of research. We developed an activated charcoal filter which traps p-BSQ from the main smoke stream. The smoke emitted from this newly developed charcoal filter does not cause protein damage or emphysema in a guinea pig model. The cigarette filter has also been patented (US Patent 2006) and is being patented in different countries of the world. Dr Mashelkar and all of us had great hopes that the cigarette filter would be readily commercialised and we would be handsomely rewarded, as our charcoal filter cigarette would be a safer cigarette that would probably not produce lung cancer or emphysema, the two fatal diseases known to be caused mostly by cigarette smoking. We were further excited when we heard that a very big multinational cigarette company was seriously considering licensing our patent. Unfortunately, we did not realise that the company was actually trying to find some commercial loopholes to circumvent our patent.

9. Vitamin C prevents cigarette smoke-induced emphysema

Emphysema is a major and increasing global cause of mortality and morbidity. It is projected to become the third most common cause of death and the fifth most common cause of disability in the world by the year 2020 (Lopez and Murray 1998; Pawels and Rabe 2004). There is no novel or even currently effective treatment aimed at preventing this irreversible, fatal disease. Although cigarette smoking is the commonest cause of emphysema, the cellular and molecular mechanisms of the disease are not yet clear. Recently, we have delineated the mechanism of cigarette smoke-induced emphysema and its prevention. This was possible only because we could produce emphysematous lung damage in

a guinea pig model within 2 weeks of cigarette smoke (CS) exposure (Banerjee *et al.* 2008). This is in contrast to reports from other laboratories, where it took at least 4–6 months of smoke exposure to produce appreciable lung damage (Banerjee *et al.* 2008). The trick was simple. Since vitamin C prevents CS-induced protein damage, which is the initial event in CS-induced emphysema, we subjected the guinea pigs to a vitamin C-restricted diet and then exposed them to cigarette smoke. Using the guinea pig model of emphysema developed in our laboratory, we demonstrated that exposure of guinea pigs to CS results in progressive protein damage, which is accompanied by apoptosis and emphysematous lesions (Banerjee *et al.* 2008). We have further demonstrated that administration of a moderately large dose of vitamin C (15 mg vitamin C/guinea pig/day) almost completely prevents protein damage and thereby prevents the subsequent events of apoptosis and emphysema (Banerjee *et al*. 2008). In smoke-exposed guinea pigs, a moderately large dose of vitamin C (15 mg/day) is needed to maintain an adequate tissue level of vitamin C. This is because cigarette smoke consumes vitamin C and a maintenance dose of 1 mg/day or 5 mg/day is inadequate to maintain sufficient tissue vitamin C content after exposure to smoke.

10. Epilogue

Vitamin C is ubiquitous. It is found in almost all plants and animal kingdoms, where its precise role is poorly understood. Till date, there has been much controversy about the exact role of the vitamin in human health. Linus Pauling argued that ascorbate could prevent or cure heart disease, stroke, cancer and infections. Conventional experts disagreed. The only role of vitamin C that has been categorically established is to prevent and cure scurvy. Reports from various laboratories of the world indeed indicate that vitamin C has a more versatile role than merely to prevent scurvy. An extensive and often confusing literature exists on the use of vitamin C in cancer. Following Pauling's work, the dietary use of vitamin C has been proposed in cancer prevention for several years. Recent pharmacokinetic data suggest that pharmacological concentrations of vitamin C can be achieved by intravenous injections rather than dietary intake. Since high concentrations of vitamin C exhibit anti-cancer activities *in vitro*; this raises the controversial question of re-evaluation of vitamin C in cancer treatment (Verrax and BucCalderon 2008). We are trying to develop an animal model of CS-induced cancer and examine the efficacy of large doses of vitamin C. Nevertheless, our results obtained with guinea pigs demonstrate that moderately large doses of vitamin C prevent cigarette smoke-induced emphysema. Since emphysema is irreversible and incurable, a practical approach should be to prevent it. Undoubtedly, the best way to prevent emphysema is to stop smoking. However, cigarette smoking is extremely addictive and various approaches to the cessation of smoking have had limited success. Therefore, if the results obtained with guinea pigs are extrapolated to humans, a regular intake of about 2 g vitamin C/day should protect a moderate smoker (10–15 cigarettes/day) from emphysema.

I have been doing research on vitamin C for the past 55 years (figure 7). At the fag end of my career, I know that even after the 80th anniversary of the discovery of vitamin C, the true picture of the biological function of this

Figure 7. I. B. Chatterjee with some of his present coworkers involved in research on the biological function of vitamin C.

outstanding vitamin is yet to be uncovered. I have come to realise that "the more I know, the more I feel my ignorance and the more I feel how much remains unknown". We still do not know why only 15% of smokers are afflicted with cancer of the lungs and other organs, and 85% are not. We are working on the hypothesis that there are presumably two distinct determining factors: (i) a genetic predisposition and (ii) a nutritional (vitamin C) deficiency. Only future research will provide the answers.

Acknowledgements

Figure 1 is from *http://en.wikipedia.org/wiki/James_Lind_(physician)*, figure 2a from History of Medicine (National Library of Medicine, USA) and figure 2b from *http://en.wikipedia.org/wiki/Charles_Glen_King*.

References

Banerjee S, Chattopadhyay R, Ghosh A, Koley H, Panda K, Roy S, Chattopadhyay D and Chatterjee I B 2008 Cellular and molecular mechanisms of cigarette smoke-induced lung damage and prevention by vitamin C; *J. Inflammation* **5** 21

Barnes P J, Shapiro S D and Pauwels R A 2003 Chronic obstructive pulmonary disease: molecular and cellular mechanisms; *Eur. Respir. J.* **22** 672–688

Bublitz C, Grollman A P and Lehninger A L 1958 The enzymic conversion of D-glucuronate to L-ascorbate and L-xylulase in animal tissues; *Biochim. Biophys. Acta* **27** 221–222

Bublitz C and Lehninger A L 1959 The enzymic conversion of L-gulonate to L-ascorbate by rat-liver enzymes; *Biochim. Biophys. Acta* **32** 290–291

Carpenter K J 1988 *The history of scurvy and vitamin C* (Cambridge: Cambridge University Press)

Chatterjee I B, Ghosh N C, Ghosh J J and Guha B C 1957 Effect of cyanide on biosynthesis of ascorbic acid in vitro; *Science* **126** 608–609

Chatterjee I B, Ghosh J J, Ghosh N C and Guha B C 1958 Effect of cyanide on the biosynthesis of ascorbic acid by an enzyme preparation from goat liver tissue; *Biochem. J.* **70** 509–515

Chatterjee I B, Ghosh J J, Ghosh N C, Chatterjee G C and Guha B C 1959a Identification of 2,keto-L-gulonolactone as an intermediate in the biosynthesis of L-ascorbic acid; *Naturwissenchaften* **46** 475–476

Chatterjee I B, Ghosh J J, Ghosh N C and Guha B C 1959b The enzyme reaction pattern in the oxidation of L-gulonolactone to L-ascorbic acid; *Naturwissenchaften* **46** 580–581

Chatterjee I B, Ghosh N C and Guha B C 1960 Biological synthesis of L-ascorbic acid in animal tissues: Conversion of L-gulonolactone into L-ascorbic acid; *Biochem. J.* **74** 193–203

Chatterjee I B, Chatterjee G C, Ghosh N C, Ghosh J J and Guha B C 1960 Biological synthesis of L-ascorbic acid in animal tissues: conversion of D-glucuronolactone and L-gulunolactone intoL-ascorbic acid; *Biochem. J.* **76** 279–292

Chatterjee I B, Kar N C, Ghosh N C and Guha B C 1961a Aspects of ascorbic acid biosynthesis in animals; *Ann. N.Y. Acad. Sci.* **92** 36–56

Chatterjee I B, Kar N C, Ghosh N C and Guha B C 1961b Biosynthesis of L-ascorbic acid: missing steps in animals incapable of synthesizing the vitamin; *Nature (London)* **192** 163–164

Chatterjee I B, Price H A and McKee R W 1965 Biosynthesis of L-ascorbic acid in different subcellular fractions of prenatal and postnatal rat livers; *Nature (London)* **207** 1168–1170

Chatterjee I B 1973 Evolution and the biosynthesis of ascorbic acid; *Science* **182** 1271–1272

Chatterjee I B, Majumder A K, Nandi B K and Subramanian N 1975 Synthesis and some major functions of vitamin C in animals; *Ann. N. Y. Acad. Sci., Second vitamin C Conference* **258** 24–47

Chatterjee I B 1978 Ascorbic acid metabolism; *World Rev. Nutr. Diet.* **30** 69–80

Chatterjee I B and Burma D P 2004 Biresh Chandra Guha – Father of modern biochemistry in India; *Curr. Sci.* **87** 823–830

Chatterjee I B 2008 Evolutionary loss of vitamin C synthesizing ability in terrestrial vertebrates; *Sci. Cult.*(in press)

Choudhary C R and Chatterjee I B 1969 Biosynthesis of ascorbic acid in birds phylogenetic trend; *Science* **164** 435–436

Ghosh M K, Chattopadhyay D J and Chatterjee I B 1996 Vitamin C prevents oxidative damage; *Free Radical Res.* **25** 173–179

Graham J B, Dudley R, Agullar N M and Gans C 1995 Implications of the late palaeozoic oxygen pulse for physiology and evolution; *Nature (London)* **375** 117–120

Grollman A P and Lehninger A L 1957 Enzymic synthesis of L-ascorbic acid in different animal species; *Arch. Biochem. Biophys.* **69** 458–467

Hassan M U L and Lehninger A L 1956 Enzymatic formation of ascorbic acid in rat liver extracts; *J. Biol. Chem.* **223** 123–138

Horowitz H H and King C G 1953a The conversion of glucose-6-C14 to ascorbic acid by the albino rat; *J. Biol. Chem.* **200** 125–128

Horowitz H H and King C G 1953b Glucuronic acid as a precursor of ascorbic acid in the albino rat; *J. Biol. Chem.* **205** 815–821

Kar N C, Chatterjee I B, Ghosh N C and Guha B C 1962 Further observation on the intracellular location and mechanism of action of the liver enzymes catalyzing the synthesis of L-ascorbic acid; *Biochem. J.* **84** 16–25

King C G and Waugh W A 1932 The chemical nature of vitamin C; *Science* **75** 357–318

King C G 1961 Introductory remarks: the history and present status of vitamin C; *Ann. N.Y. Acad. Sci.* **92** 3–5

Lehninger A L 1978 *Biochemistry*, Fiirst Indian edition (New Delhi: Kalyani Publishers)

Lind J 1753 *A treatise of the scurvy* (Edinburgh: Miller)

Lopez A D and Murray C C 1998 The global burden of disease, 1990–2020; *Nat. Med.* **4** 1241–1243

McCord C P 1959 Scurvy as an occupational disease; *J. Occup. Med.* **1** 315–318

Mukhopadhyay C K and Chatterjee I B 1994 NADPH-initiated cytochrome P450-mediated free metal ion-independent oxidative damage of microsomal proteins: exclusive prevention by ascorbic acid; *J. Biol. Chem.* **269** 13390–13397

Muhkopadhyay C K and Chatterjee I B 1994 Free metal ion-independent oxidative damage of collagen: protection by ascorbic acid; *J. Biol. Chem.* **269** 30200–30205

Nandi A, Mukhopadhyay C, Ghosh M K, Chattopadhyay D J and Chatterjee I B 1997 Evolutionary significance of ascorbic acid synthesizing capacity in terrestrial vertebrates; *Free Radical Biol. Med.* **22** 1047–1054

Nishikimi M, Fukuyama R, Minoshima S, Shimizu N and Yagi K 1994 Cloning and chromosomal mapping of the human nonfunctional gene for L-gulonolactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man; *J. Biol. Chem.* **269** 13685–13688

Padayatti S J, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, Chen S, Corpe C, Dutta A, Dutta S K and Levine M 2003 Vitamin C as an antioxidant: evaluation of its role in disease prevention; *J. Am. Coll. Nutr.* **22** 18–35

Panda K, Chattopadhyay R, Ghosh M K, Chattopadhyay D J and Chatterjee I B 1999 Vitamin C prevents cigarette smoke induced oxidative damage of proteins and increased proteolysis; *Free Radical Biol. Med.* **27** 1064–1079

Panda K, Chattopadhyay R, Ghosh M K, Chattopadhyay D J and Chatterjee I B 2000 Vitamin C prevents cigarette smoke induced oxidative damage in vivo; *Free Radical Biol. Med.* **29** 115–124

Pauling L 1970 *Vitamin C and the common cold* (California, USA: W H Freeman and Company)

Pawels R A and Rabe K F 2004 Burden and clinical features of chronic obstructive pulmonary disease (COPD); *Lancet* **364** 613–620

Pryor W A, Stone K, Zang L-Y and Bermudez E 1998 Fractionation of aqueous cigarette tar extracts: fraction that contain the tar radical cause DNA damage; *Chem. Res. Toxicol.* **11** 441–448

Scrimshaw N S and Young V R 1976 The requirements of human nutrition; *Sci. Am.* **235** 1168–1170

Svirbely J L and Szent-Györgyi A 1932 Hexuronic acid as the antiscorbutic factor; *Nature (London)* **129** 576

Szent-Györgyi A 1928 Observations on the function of peroxidase systems and the chemistry of the adrenal cortex; *Biochem. J.* **22** 1387–1409

Szent-Györgyi A 1963 Lost in the twentieth century; *Annu. Rev. Biochem.* **32** 1–14

Szent-Györgyi A 1970 *Vitamin C and common cold* (California: Linus Pauling, Freman and Company) p. 36

US Patent No. 6,929,012, dated August 16, 2005. *Process for the isolation of a major harmful oxidant from cigarette smoke* (Inventor: Indu Bhusan Chatterjee, Assignee: CSIR)

US Patent No. 7,025,067 B2, dated April 11, 2006. *Activated charcoal filter for effectively reducing p-benzosemiquinone from the mainstream cigarette smoke* (Inventor: Indu Bhusan Chatterjee, Assignee: CSIR)

Verrax J and Buc Colderon P 2008 The controversial place of vitamin C in cancer treatment; *Biochem. Pharmacol.* **76** 1644–1652

Young F G 1960 Editorial; *Br. Med. J.* **1** 1871

*e*Publication: 27 May 2009