Collagen and scurvy
Understanding the connection

Eliana Lingard

Scurvy was a common disease among sailors in the eighteenth century. It is now known to be caused by vitamin C deficiency. Postgraduate researcher Eliana Lingard explains how scurvy, vitamin C and the protein collagen are linked

Exam links

AQA Proteins
Edexcel A Proteins; Core practical 2
Edexcel B Proteins
OCR A Biological molecules
OCR B Proteins and enzymes
WJEC Eduqas Proteins

When asked to give an example of a disease, people often suggest those caused by microorganisms. But diseases can also be linked to lifestyle factors, such as smoking and diet. A major disease in the eighteenth century was scurvy, caused by vitamin C deficiency. Research shows that many of its symptoms are due to defects in a protein called collagen.

Collagen: holding it together

Collagen is found in nearly all our tissues and is crucial for life. It accounts for about one-third of our total protein and is the major structural protein in skin, blood vessels, ligaments, cartilage, bone and teeth. As a key part of **connective tissue**, collagen provides **tensile strength**, bulk and support to tissues and organs. Collagen also provides binding sites that help cells attach to, and interact with, their surroundings, which in turn dictates tissue structure and supports tissue repair and wound healing.

Because collagen is so important and ubiquitous, alterations to its structure or the incorrect assembly of its molecules can have serious impacts on our health. This is demonstrated by several connective tissue diseases that can severely reduce a person's

Legs of a scurvy sufferer. Haemorrhages are attributed to abnormal blood vessels, which, together with hard lumps on the muscles, are caused by defective collagen

Table 1 Genetic diseases involving collagen

Disease	Cause	Symptoms
Osteogenesis imperfecta (brittle bone disease)	Impaired collagen production	Fragile bones that break easily Altered bone structure Short stature Dental problems
Ehlers-Danlos syndrome	Disrupted collagen production and assembly	Loose joints (hypermobility) Elastic, fragile skin Muscle fatigue
Alport syndrome	Incomplete collagen formation in the basement membrane	Eye abnormalities Hearing loss Progressive kidney failure

quality of life, or even prove fatal (see Table 1). Scurvy is an example of a disease in which connective tissue function is compromised because of defective collagen assembly. Whereas some collagen is long-lived (tendon, cartilage, bone), defective collagen assembly affects tissues in which collagen must be continuously made and replaced (**basement membrane**, blood vessel wall, skin). After just a few months of disrupted collagen formation, severe symptoms of scurvy can appear. These include tooth loss, bleeding gums, poor wound healing and easy bruising. Scurvy may appear in people with poor diets, such as a lack of fruit and vegetables over the winter months.

Unravelling collagen structure and assembly

Under the microscope, collagen appears as long, thin fibres that are bundled together or spread out to form branched networks. The fibres, when stained, contain alternating dark-and-light bands because they are composed of multiple, staggered collagen molecules (see Figure 1).

The organisation and abundance of collagen varies between tissues, giving them their specific mechanical properties and structures. For example, in tendons, the unidirectional alignment of bundled collagen fibres gives them their tensile strength and **resilience**. In contrast, collagen filaments surrounding blood vessels are arranged randomly in networks that resist tension from multiple directions. The network supports the blood vessel by providing resistance to blood pressure and by separating it from the surrounding tissue. In bones, calcium phosphate crystals added to orderly layers of collagen make the fibres more rigid, increasing their stiffness and strength.

Collagen is a protein and its functions are governed by its shape. Therefore, it is important that we understand how collagen fibres are assembled. Collagen's assembly into its final, fibrous structure can be broadly represented by the different levels of protein structure (see Figure 2). There is currently no agreement between scientists on whether collagen has a tertiary structure. Since three helical polypeptide chains associate with each other to form a single collagen molecule, it can be argued that the collagen alpha helix is both a secondary and tertiary structure.

Collagen molecules form inside cells. They are rope-like quaternary protein structures consisting of three alpha-helix chains wound tightly together to form a stable triple helix. After leaving the cell, the collagen molecules self-assemble to form long collagen fibrils, which are stabilised by covalent cross-links between adjacent molecules. These cross-links are generated by specific amino acids within the collagen molecules. The fibrils aggregate side-by-side with other triple helix collagen molecules to form collagen fibres hundreds of micrometres long and several micrometres across. These then arrange to form structures capable of resisting tensile forces.

Collagen's amino acids

The primary structure of collagen is important for collagen assembly, and hence its function. Collagens are long sequences of the repeated amino acid sequence glycine–X–Y, where X is usually proline and Y is a modified version of proline called hydroxyproline. Hydroxyproline has a hydroxyl (–OH) group added to the fourth carbon atom in proline's functional group (see Figure 3). This modification is called hydroxylation and is vital for collagen assembly. Hydroxyproline residues in

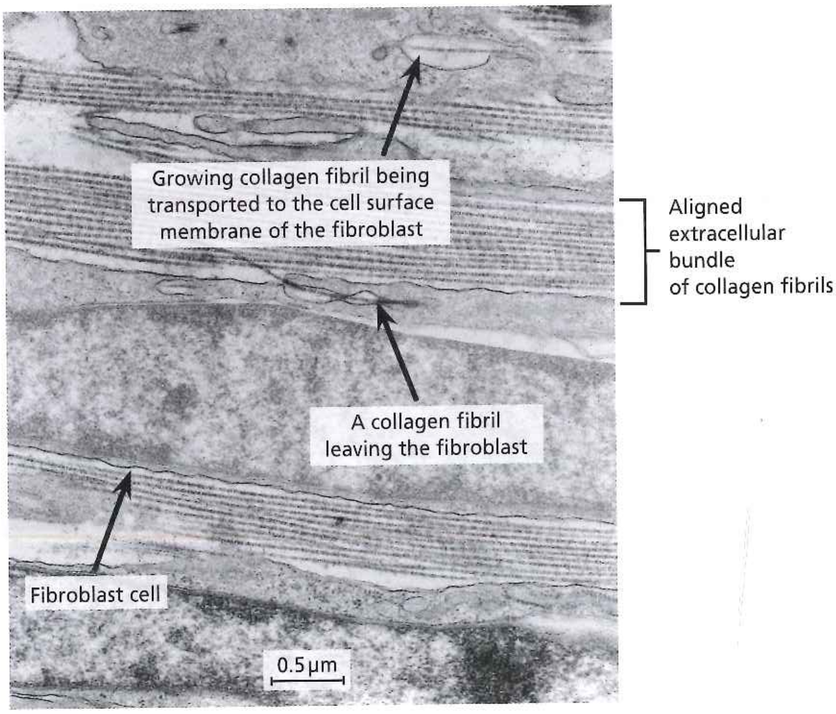


Figure 1 Transmission electron micrograph showing collagen fibril deposition and organisation. Collagen is synthesised inside fibroblasts. The fibrils are then transported out of the fibroblast via specialised cell surface membrane channels

Terms explained

Basement membrane A two-dimensional mesh of proteins that separates cells from connective tissue.

Cofactor A non-protein compound that is necessary for an enzyme's activity.

Connective tissue Fibrous tissue that supports, protects and connects other tissues in the body.

Resilience The ability of a material to return to its original shape after being deformed.

Tensile strength The resistance to being stretched.

Box 1 James Lind's investigation

James Lind carried out a trial using six pairs of sailors afflicted with scurvy, to see whether acidic substances could cure them. The pairs were given either cider, vinegar, dilute sulfuric acid, sea water, purgative paste or two oranges and a lemon to eat daily. Although the citrus fruits ran out after 6 days, ending the trial, Lind found that the two sailors who were given them recovered quickly.

Lind's study had a number of weaknesses. It used a small number of subjects, failed to record the concentrations of the mixtures given, and ended prematurely when the fruit ran out. However, Lind showed awareness that non-treatment-related factors could interfere with his experiment. In addition to keeping each treatment type and dosage constant, Lind tried to keep other variables constant by selecting sailors who:

- all had scurvy
- came from the same quarter of the ship
- shared the same basic diet

Despite its limitations, Lind's study is significant because it is one of the first **controlled** clinical trials on record (see Biological Sciences Review, Vol. 32, No. 1, p. 19).

neighbouring polypeptide chains form hydrogen bonds with each other that stabilise the triple helix structure.

Proline and glycine also help stabilise the triple helix. Proline's rigid structure enables the polypeptides to wrap around each other. Glycine's small side chain, which is a single hydrogen atom, allows the polypeptide chains to associate closely with each other, promoting hydrogen bond formation. Together, these amino acids make collagen molecules rigid and strong.

The name's C, Vitamin C

Scurvy was common among sailors in the eighteenth century. In 1747, naval surgeon James Lind carried out a trial to investigate whether any of the scurvy remedies used by sailors at the time were effective (see Box 1). Lind found that giving sailors citrus fruits successfully treated scurvy. This finding led to the eventual eradication of scurvy among naval personnel. Although Lind didn't know it, oranges and lemons cure scurvy because they contain vitamin C.

Hydroxylation

Scurvy is caused by a lack of vitamin C — ascorbic acid — an essential nutrient for humans. Vitamin C can only be obtained from our diet as our bodies cannot synthesise it. Any vitamin C that isn't used is quickly excreted in our urine, so we need to consume it daily. When people don't have enough vitamin C in their diet for several months, they develop scurvy. Vitamin C deficiency causes scurvy because it is a **cofactor** for the enzyme that catalyses amino acid hydroxylation (see Figure 4). A cofactor is a non-protein chemical compound that is not itself an enzyme but must be present for an enzyme to work. Vitamin C is a cofactor for the enzyme proline

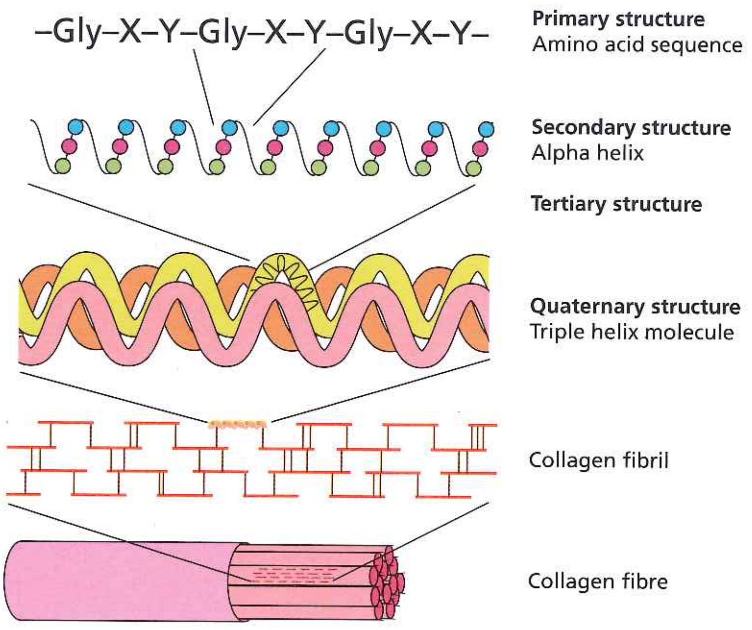


Figure 2 The assembly and organisation of collagen protein

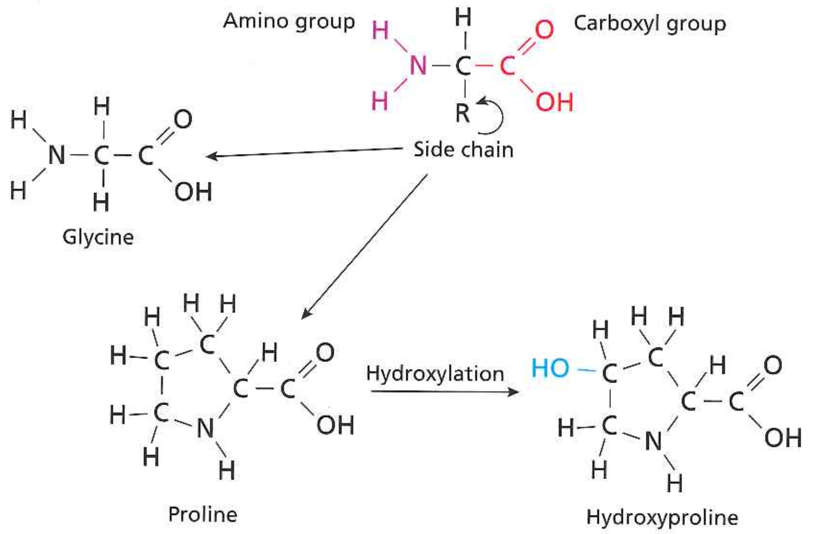


Figure 3 Structures of the three main amino acids in collagen. The basic structure of an amino acid is shown at the top

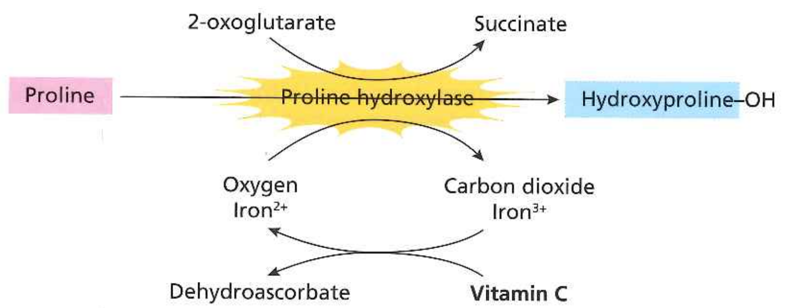
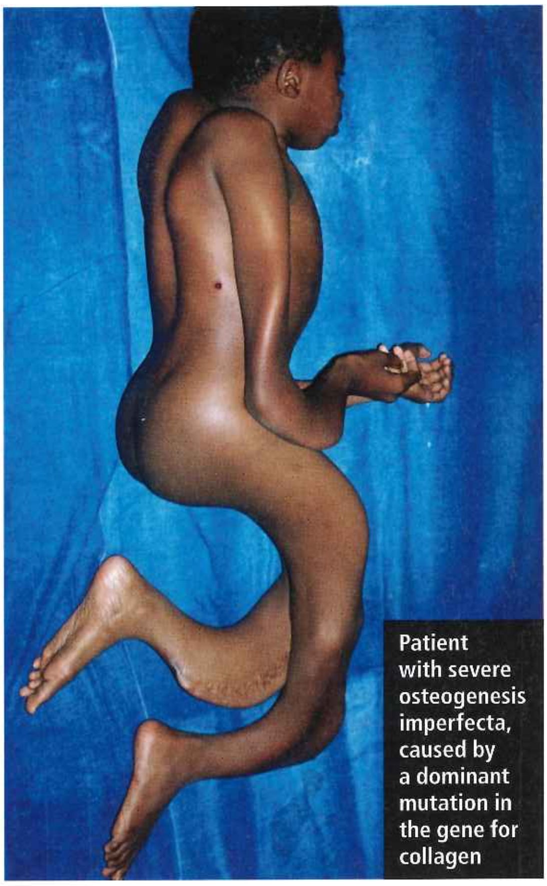


Figure 4 The enzyme proline hydroxylase needs an electron from vitamin C to keep it functional

Patient with Ehlers-Danlos syndrome. The elasticity of the skin is caused by disrupted collagen synthesis



Patient with severe osteogenesis imperfecta, caused by a dominant mutation in the gene for collagen

hydroxylase, which catalyses the addition of hydroxyl groups to proline to form hydroxyproline.

After collagen translation, proline hydroxylase binds to proline-rich areas of the polypeptide. Oxygen, iron and a biological acid called 2-oxoglutarate donate and accept electrons and chemical groups to enable the reaction. This allows proline hydroxylase to make the hydroxyl group and attach it to proline's side chain. Once proline has been hydroxylated, the donation of an electron from vitamin C to iron keeps the active site of proline hydroxylase functional (see Figure 4).

Lysine residues in collagen can also be hydroxylated, catalysed by the enzyme lysine hydroxylase. This enzyme again requires vitamin C as a cofactor. Hydroxylation of lysine promotes cross-linking of collagen molecules to form stable fibrils, which further strengthens the collagen fibres.

Losing strength

Because it is essential for amino acid (especially proline) hydroxylation, vitamin C is crucial for collagen assembly. Without vitamin C, collagen stability is compromised. Less hydroxyproline means less hydrogen-bonding between collagen's polypeptide chains. Less lysine hydroxylation reduces collagen fibril cross-linking. Collagen molecules that lack sufficient hydrogen bonds cannot maintain their structure and degrade. Since collagen fibres frequently need to be repaired due to wear and tear, long-term disruption to collagen production means that the collagen fibres become weak. As a result, tissues lose their mechanical strength and support.

When people lack sufficient vitamin C, worn-out collagen fibres in their bodies are not adequately replaced. Connective tissues lose the strength and resilience to perform functions such as anchoring teeth to gums, maintaining tendon structure, and supporting blood vessels. Symptoms including bleeding gums, bruising, tooth loss, weak bones and slow wound healing develop. If left untreated,

Exam style questions

1 Name two types of chemical bond that stabilise a collagen molecule. [1 mark]

2 Explain why scientists agree that collagen has a primary, a secondary and a quaternary structure but not whether it has a tertiary structure. [3 marks]

3 Suggest how vitamin C deficiency might affect ultrafiltration in a human kidney. Explain your answer. [3 marks]

BiologicalSciencesReview*Extras*

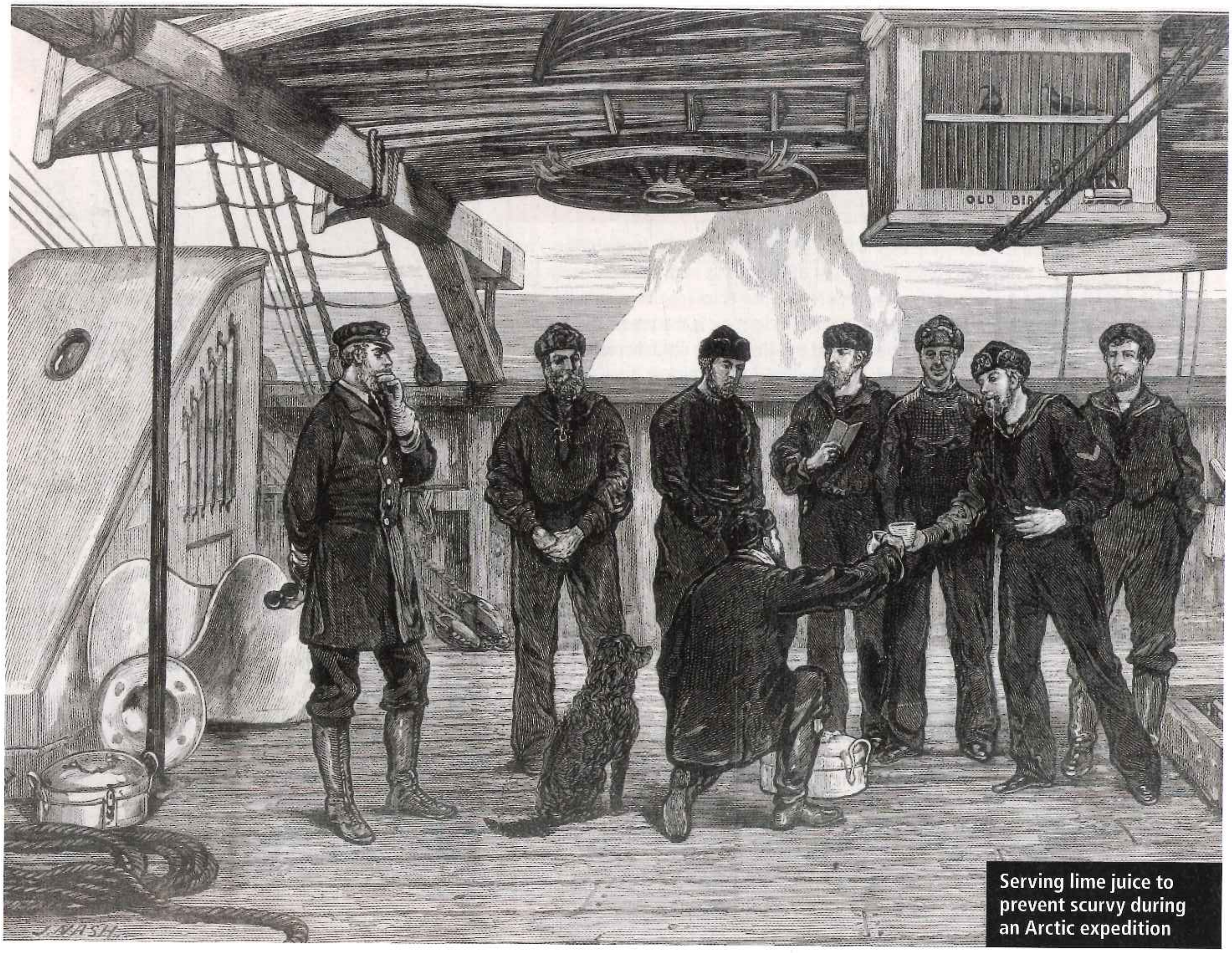
Go online for the answers at www.hoddereducation.co.uk/bioreviewextras

Further reading

American Chemical Society National Historic Chemical Landmarks, 'Albert Szent-Györgyi's discovery of vitamin C': **https://tinyurl.com/zmewd62**

Science Daily, 'Study links changes in collagen to worse pancreatic cancer prognosis': **https://tinyurl.com/hpga83c**

The James Lind Library: **www.jameslindlibrary.org**


Serving lime juice to prevent scurvy during an Arctic expedition

the continued loss of collagen and compromised organ functions lead to death.

Discovery of vitamin C

Finding a cure for scurvy was the first step to understanding the role vitamin C plays in collagen assembly and function. James Lind's findings in 1747 underpinned the work of several scientists whose collective research led to the discovery of vitamin C. In 1907, Axel Holst and Theodor Frølich identified nutritional deficiency as the cause of scurvy. This led to the isolation and identification of vitamin C in 1930 by Albert Szent-Györgyi and J. L. Svirbley. By 1933, the chemist Norman Haworth had deduced its chemical structure. Since then, biologists, physicists, chemists and doctors have worked together to decipher the molecular and biochemical events in which vitamin C is involved. Now we know how scurvy develops and why. Because of this, a serious disease that once affected millions of people is now extremely rare.

Building on previous discoveries is an important part of being a scientist. In our research, we apply what we already know to solve new problems. Using our knowledge of collagen structure and function, scientists have started investigating the role collagen plays in a range of different disorders, including the development of cancer.

Topics for discussion

- Design an experiment to investigate cures for scurvy that would overcome the limitations encountered by Lind.
- In modern, Western society, who might be at risk of developing scurvy?

Eliana Lingard is studying for a PhD in mechanobiology at The University of Manchester. She investigates how cells detect and respond to forces generated by their surroundings. Her research explores new biomaterials that mimic dense breast tissue, for use in experiments investigating breast cancer.

Key points

- Scurvy is a disease caused by vitamin C deficiency.
- Vitamin C is crucial for the assembly of the structural protein collagen.
- Collagen gives tissues support, structure and strength.
- When collagen is not formed properly due to vitamin C deficiency, the structure of tissues is compromised.