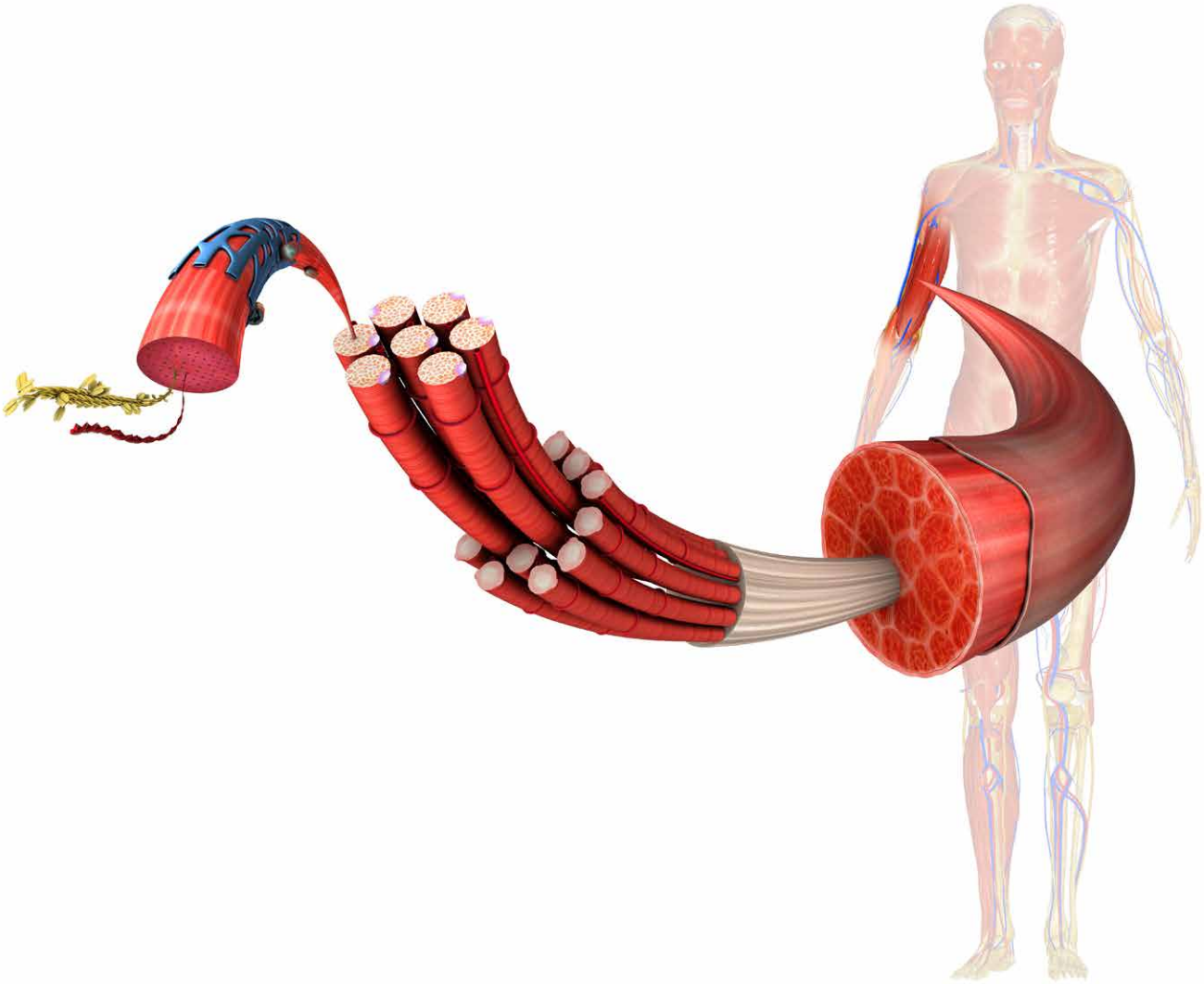
The Role of Nitrate and Nitric Oxide in Muscle Blood Flow in Exercise

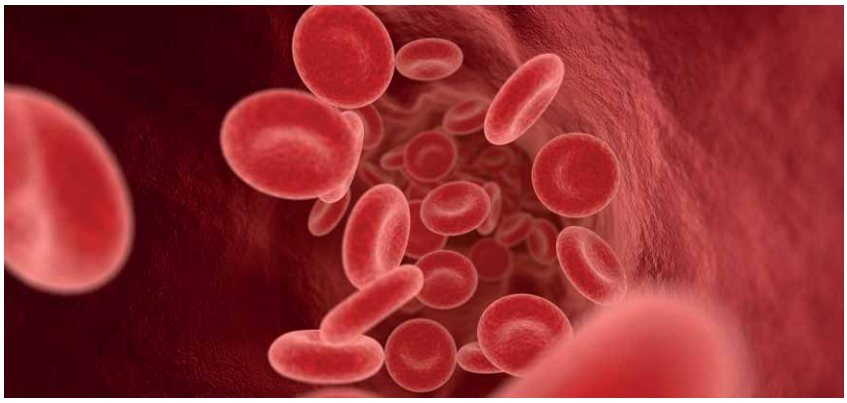
Dr Alan N. Schechter



Scientia

THE ROLE OF NITRATE AND NITRIC OXIDE IN MUSCLE BLOOD FLOW IN EXERCISE

Nitric oxide is an 'A-list' celebrity amongst chemical compounds. Proclaimed 'molecule of the year' in 1992 by the American Association for the Advancement of Science, its physiological importance, discovered in 1985, was recognised in 1998 by the award of a Nobel prize to some of the researchers who had discovered its vital role in regulating blood vessels and blood pressure. Here we look at the pre-eminent work of **Dr Alan N. Schechter** at the National Institutes of Health in Bethesda, Maryland, USA, that is continuing to keep nitric oxide at the front and centre of groundbreaking biological research.



Nitric Oxide

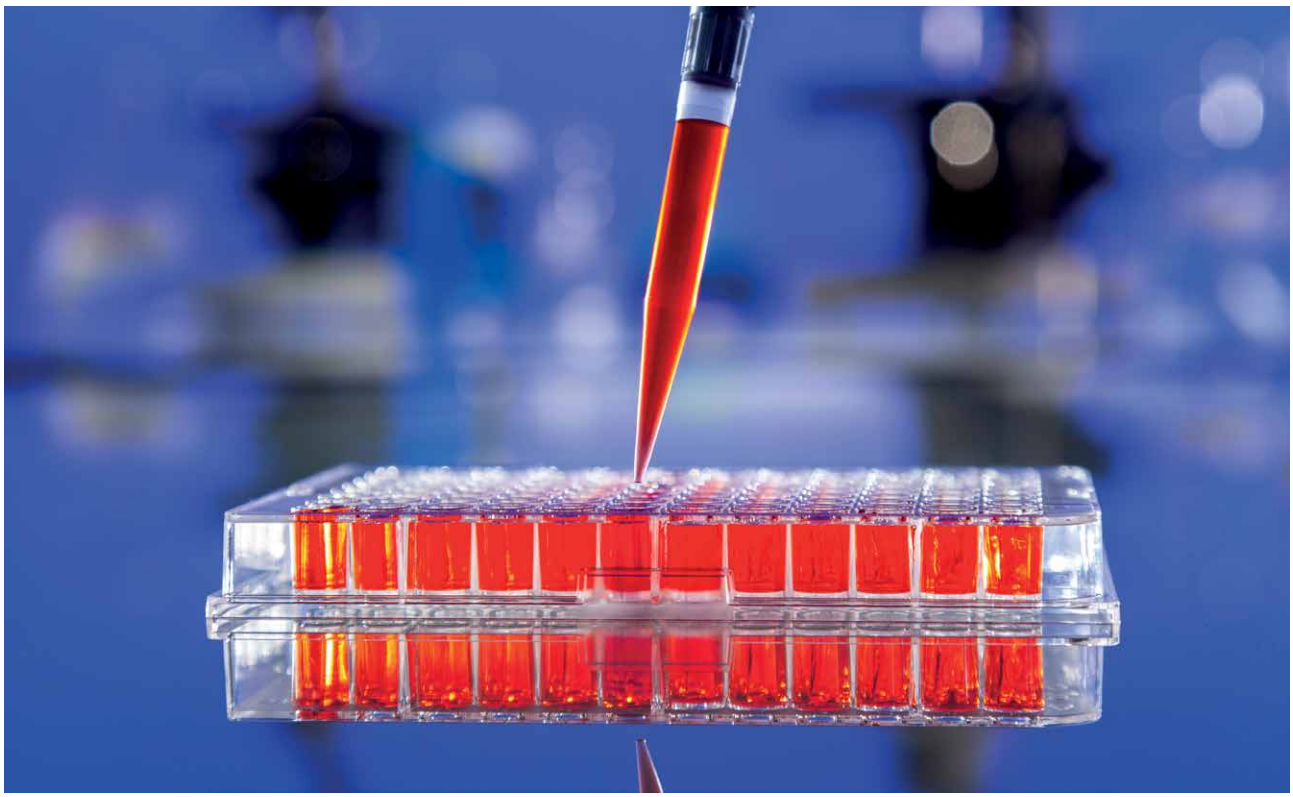
Nitric oxide is a colourless gaseous oxide of nitrogen and is a free radical, meaning that it has an unpaired electron, which is relatively uncommon in chemistry. Historically, nitric oxide was generally considered to be only an air pollutant, a by-product of fossil-fuel combustion. However, research in the late 1980s and early 1990s indicated that nitric oxide has a central role in virtually all cells and, in particular, in animal physiology as well as pathophysiology, the processes associated with disease or injury.

High-profile research on nitric oxide has captured the attention of the scientific world for more than a third of a century, resulting from the pivotal discovery of its role as a cardiovascular signalling molecule, regulating blood pressure and maintaining the health and function of blood vessels. Although nitric oxide is toxic at high concentrations in mammals, including humans, it acts at very low concentrations as a critical signalling or messenger molecule. Its small size enables it to pass easily through cell membranes and walls to carry out various signalling functions. The free radical state of the molecule ensures that it is more reactive than other cellular signalling molecules but limits its chemical lifetime, especially in the presence of oxygen or macromolecules.

Nitric Oxide Metabolism and Transport

Since the early 2000s, there has been a renewed program of research on nitric oxide, some of it emanating from the laboratory of Dr Alan N. Schechter in its Molecular Medicine Branch at the National Institutes of Health (NIH) in Bethesda, Maryland, USA.

Dr Schechter's laboratory has a particular interest in how nitric oxide interacts with haemoglobin, a protein that carries oxygen from the lungs to the rest of the body. Working with a multidisciplinary research team, he has been investigating how nitric oxide is formed and transported by blood, and its potential for use as a pharmacological agent to help deliver treatment (i.e., act as a drug). Dr Schechter believes that using nitric oxide in this way may contribute to the development of therapies for diseases such as sickle cell anaemia and others with impaired blood flow and thus diminished oxygen transportation. One outcome of this work is that Dr Schechter is a co-inventor on a patent at the NIH for the therapeutic uses of nitrite ions (precursors of nitric



oxide), which has been licensed for development by several companies.

Emerging from this line of research, new concepts have been developed in the last two decades on new pathways of formation (as described below) and how these pathways modulate platelet reactivity and blood clotting, and, most recently, how nitrate ions determine muscle function and blood flow.

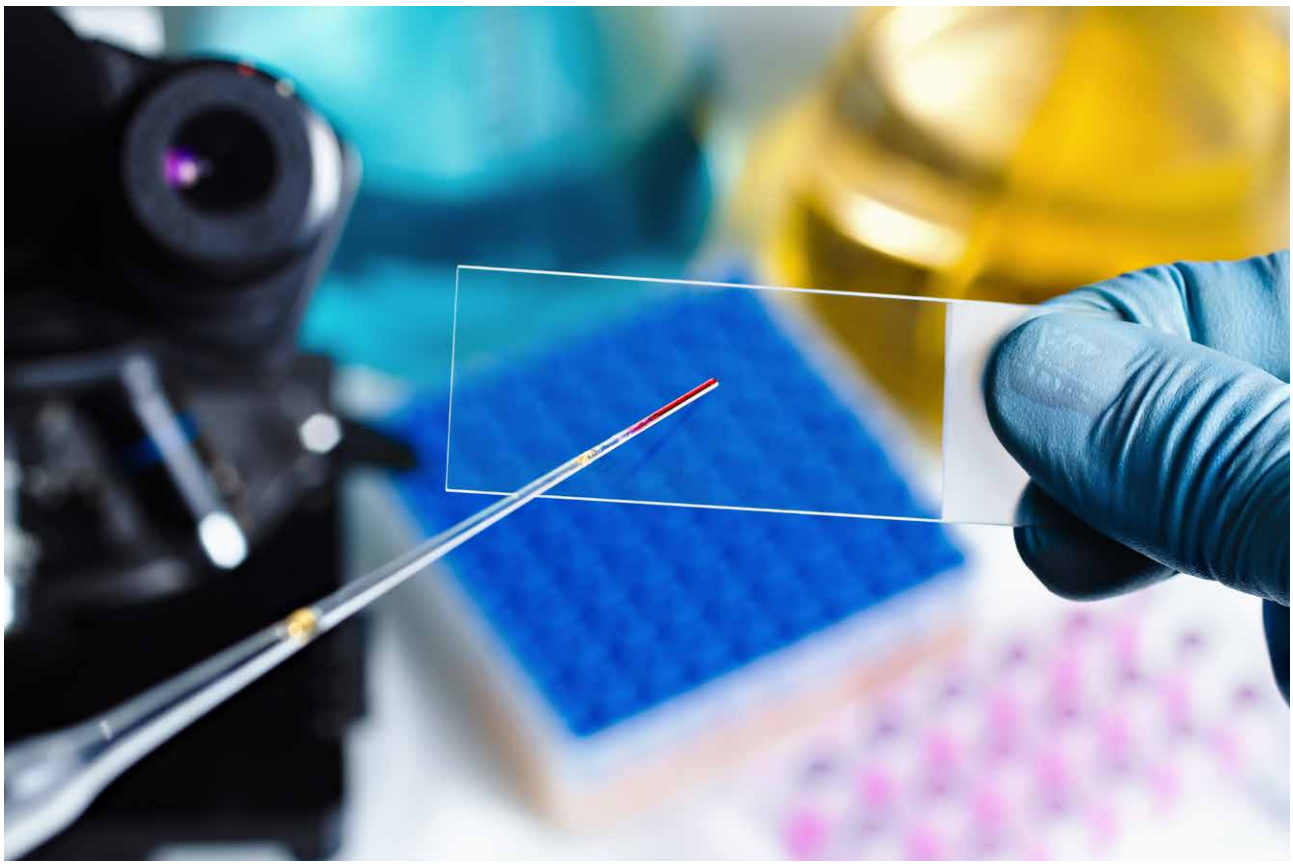
Nitric Oxide Formation

In order to support its broad range of functions and effects in mammals, nitric oxide is produced by tissues in the body in a number of ways. Over the past two decades, Dr Schechter has led a wide-ranging group of researchers to explore these issues in greater depth.

Working closely with Dr Mark Gladwin and many others at the NIH, Dr Schechter has shown, especially under hypoxic conditions, a new pathway of nitric oxide formation in addition to that which occurs when oxygen is freely available in which nitric oxide is mainly produced from the enzymatic conversion of arginine to citrulline by nitric oxide synthase enzymes. Over the past 15 years, these investigators have shown that nitric oxide formation in the blood by the reduction of nitrite ions to nitric oxide by red cell haemoglobin and other proteins and reducing agents, reflects very important sources of nitric oxide production, especially when oxygen is limited. It has been discovered that the cycling of nitric oxide formation and destruction (also by heme proteins such as haemoglobin) is at the heart of the regulation of diverse physiological properties, of which probably the most important is to control the vascular tone of blood vessels and arteries – and thus blood flow.

In their most recent work, conducted with Dr Barbora Piknova in particular, the researchers have shown that in muscle, when oxygen supplies are reduced, such as during exercise, nitric oxide can be supplied by various nonenzymatic reactions, resulting especially from the reduction of the high concentrations of nitrate found in muscle tissues, as well as from nitrite reduction. It is important to notice that this whole reduction cycle from nitrate to nitrite and nitric oxide is innate to mammalian cells that process the entire enzymatic/transporting machinery necessary for maintaining the working cycle, as explained in more detail below.

With the recognition that nitrate and nitrite ions have a vital role in the production of nitric oxide, there has been a much greater focus on the sources and production of nitrites in the body. It was generally thought until recently that the only tissues with nitrate- and nitrite-to-nitric oxide reductase activity (i.e., the nitric oxide cycle) were the blood and some internal organs, predominantly the liver. Nitrate is generally supplied either from the diet or by the oxidation of excess nitric oxide by red cell haemoglobin in the blood. Both ions are also present in foods, especially in green leafy plants, and have long been used to preserve meat products. Dr Schechter and many others in the field believe that although there has long been some uncertainty over their safety in the diet (based on certain animal studies at very high concentrations) that the benefits of these ions in the diet far outweigh any risks.



To learn more, Dr Schechter has recently collaborated with researchers worldwide to investigate the dietary intake of nitrate and nitrite ions, particularly to better understand the metabolism of these nitric oxide precursors in skeletal muscle, a tissue largely unconsidered in this context up to this point. The general physiology of muscle tissue (especially response in exercise), has been widely understood for more than a century, particularly regarding hyperaemia, the rapid 10 to 20-fold (or greater) increase in blood flow into muscle tissue in response to increased metabolic demand for oxygen during heavy exercise. However, the detailed biochemical control of these processes has been much less understood up until now.

In particular, vasodilation or widening of the blood vessels is mediated by the synthesis and release of a range of vasodilatory agents, of which nitric oxide is just one of many (although likely the most potent). These act to relax smooth muscle cells within the walls of large veins, arteries, and arterioles, allowing greater blood flow through them. It has long been speculated that substances produced by muscles affect vasomotor tone. However, none of the potential vasodilators had all the properties necessary until nitric oxide, originally identified as endothelium-derived relaxing factor, was investigated but studies using inhibitors of the nitric oxide synthase enzymes were not consistent with the idea that nitric oxide was the major factor in the control of blood flow to muscle during exercise.

Human Skeletal Muscle is a Reservoir for Nitrate Storage

Dr Schechter's recent studies suggest that skeletal muscle may have developed control of blood flow as a vital function for its nitrate ion reservoir during exercise. In various rodents, and later confirmed in human samples by Dr Schechter's group and several other laboratories, the nitric oxide precursor, nitrate, has been found in far greater quantities in skeletal muscle than in other organs, including blood. A significant proportion of this baseline nitrate storage reservoir was observed to be produced by the nitric oxide synthase enzymes, but it is also significantly boosted from the consumption of nitrates in the diet.

This muscle stored nitrate reservoir is highly accessible via the blood circulatory system and therefore can be easily transported to internal organs (namely the liver, which is the organ with high nitrate reductase activity) to be reduced to nitrite and nitric oxide, as well as direct reduction of nitrate into nitric oxide in the muscle tissue itself. It is notable that although researchers found that the nitrate reductive activity in muscle is significantly less than that of the liver per milligram of tissue, for example, the large total mass of the muscle tissue (as the largest 'organ' in the body) means that even low levels of activity in the muscle ensure that this tissue can be the main site for the production of basal levels of nitrite and nitric oxide.

Continuous amounts, at least at low levels, of nitric oxide are produced in muscle tissue (and probably all tissues and organs) even when at rest, due to its ubiquitous requirements as a signalling molecule, but its production is dramatically

increased during muscle contraction. It has been widely observed that nitrate supplementation, in the form of beetroot juice or sodium/potassium nitrate, can reduce blood pressure and has many other physiological effects such as slightly improving athletic performance. These benefits have been attributed to an increase in the bioavailability of nitric oxide, and it has been further shown that the ingested nitrate is stored within muscle tissues, with a five-fold rise in nitrate and a three-fold rise in nitrite in muscle two hours after the consumption of beetroot juice.

The Role of Sialin and CLC-1

Sialin, a nitrate transporter protein expressed in the salivary glands has been found to be involved in the uptake of nitrate by muscle cells in culture, although as yet its role in muscle storage of nitrate is unclear. However, sialin has been found to be expressed and recruited to the sarcolemmal membrane (the cell membrane of striated muscle fibre cells) and may be involved in the uptake of nitrate from the circulatory system. Research is continuing to clarify the importance of sialin in the suggested mediating role in the storage of nitrate in skeletal muscle, and the muscle's potential function as a whole-body regulator of blood nitrate, controlling the levels circulating in the plasma. CLC-1, an anion transporter specific for skeletal muscle, had been also shown to play a role in nitrate transport into muscle cells. However, the detailed roles of these and, possibly other anion transporters, are still to be elucidated.

In the conversion of stored nitrate to bioactive nitrite and nitric oxide, research on rodents has highlighted the role of the enzyme xanthine oxidoreductase (XOR) and possibly also aldehyde oxidase (AO) in the step-by-step reduction of nitrate to nitrite and nitric oxide in numerous tissues. Both enzymes are expressed in human muscle, and they may be involved in the regulation and conversion of muscle stored nitrate during high-intensity exercise. It has been noted that nitrate levels remain high in resting muscles, but concentrations fall during high-intensity exercise, especially following supplemental nitrate consumption (when muscle nitrate content has been elevated), suggesting that tissues respond to ambient levels of these ions to maximise their physiological effects. These types of effects will be important in working out how to optimize supplementation for achieving physiological effects, including in athletic performance.

Several key strands of research emanating from Dr Schechter's focussed interest in nitric oxide are opening opportunities for a greater understanding of skeletal muscle and the central role of nitrate in exercise and blood flow. These recent studies of nitric oxide formation in muscle have, in particular, caught the attention of investigators in exercise and sports medicine, such as Dr Andrew Jones's group at the University of Exeter in the UK and have resulted in extensive cooperative research related to these fields, as well as one recent scientific symposium at Exeter on this new topic.

'A Remarkable Career of Scientific Accomplishment'

NIDDK Scientific Director Michael Krause recently stated that 'Dr Schechter has had a remarkable career of scientific accomplishment.' Even after 50 years, Dr Alan Schechter continues to be a driving force for an ever-broadening research programme into nitrates, nitrites and nitric oxide, which will have positive consequences for decades to come.

His own particular passion for developing treatments for genetic diseases of haemoglobin, including sickle-cell disease, has led to pioneering clinical research that demonstrates the value of the medication hydroxyurea in treating this disease. More recently, this work has led to a diversification of his team's research into the understanding of the role of haemoglobin in the formation and metabolism of the signalling molecule nitric oxide, and the spin-off (but no less important) research into the critical role of nitrate, nitrite ions and nitric oxide in the regulation of vasodilation, particularly in exercise, linked to its storage in skeletal muscle.

Currently, Dr Schechter is working with several ophthalmologists in also studying nitric oxide formation and function in the mammalian eye. These studies, which have primarily involved analyses of rodent and porcine eyes, have suggested that much of the nitric oxide important for many functions in the eye is also produced by reduction of nitrate ions. Further, they also suggest that the lacrimal glands function in a way analogous to the salivary glands, in using sialin and perhaps other transporters to transfer nitrate from the blood into the tears. In view of the recent use of a nitric oxide donor drug to treat glaucoma, these findings – which will soon be tested in human volunteers – may open up a new area of ocular pharmacology.

Summary

Both directly and indirectly, Dr Schechter's work has led for the first time, to baseline levels of nitrate and nitrite being discovered in far higher concentrations in skeletal muscle than in plasma, and that muscle acts as a reservoir for excess nitrate. When linked to the newly established presence of sialin in skeletal muscle, an active nitrate transporter, this suggests that sialin, together with CLC-1, may be responsible for the increase in muscle nitrate concentration observed following the ingestion of dietary nitrate. The indications are that skeletal muscle acts as a nitrate and nitrite reservoir, functioning to support whole-body nitric oxide homeostasis via a regulated distribution of these ions into the bloodstream. Lastly, the reduction in nitrate concentration following consumption of nitrate occurs following high-intensity exercise, provides the first indication that in skeletal muscle, and perhaps other muscle tissues (or even other organs), that nitric oxide is generated from nitrate stored in the muscles.

Meet the researcher

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Dr Alan N. Schechter graduated with a BA in zoology from Cornell University in 1959 and completed his MD at Columbia University in 1963. In an eminent and accomplished scientific career spanning more than 60 years, Dr Schechter has worked on protein folding with Dr C. B. Anfinsen, a Nobel Prize winner and has also pioneered the development of treatments for genetic diseases of haemoglobin, including sickle-cell disease. More recently, he has focussed on understanding the role of haemoglobin in the formation and metabolism of the signalling molecule nitric oxide. Dr Schechter is now a Senior Scientist and Chief of the Molecular Medicine Branch of the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health in Bethesda, MD. An author on more than 350 scientific papers, Dr Schechter has published in a range of prestigious international journals. Furthermore, Dr Schechter has served on the board of the Foundation for Advanced Education in the Sciences at NIH for almost half-a-century, has helped found and chair the Office of NIH History, and has served on and chaired the Council of the NIH Assembly of Scientists.

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FURTHER READING

JW Park, B Piknova, A Jenkins, Potential roles of nitrate and nitrite in nitric oxide metabolism in the eye, Scientific Reports, 2020, 10(1), 13166, doi:10.1038/s41598-020-69272-9.

LJ Wylie, JW Park, A Vanhatalo, et al, Human skeletal muscle nitrate store: influence of dietary nitrate supplementation and exercise, Journal of Physiology, 2019, 597, 5565–5576.

B Piknova, JW Park, KKJ Lam, AN Schechter, Nitrate as a source of nitrite and nitric oxide during exercise hyperemia in rat skeletal muscle, Nitric Oxide, 2016, 55–56, doi:10.1016/j.niox.2016.03.005.

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