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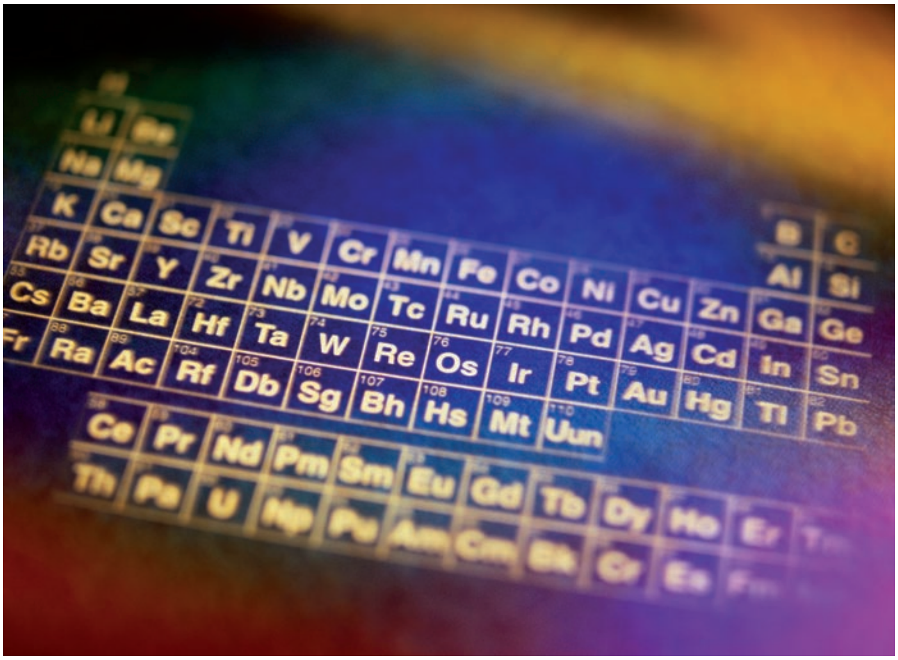
A toxic brew we cannot live without

Micronutrients give insights into the interplay between geochemistry and evolutionary biology

The physician, alchemist and astrologer Paracelsus (1493–1541)—who is widely regarded as the father of toxicology—famously wrote, "[a]ll things are poison and nothing is without poison, only the dose permits something not to be poisonous." Yet, if one believes the modern barrage of health literature, dietary advice and advertisements from a growing dietary supplement industry, humans ought to take more micronutrients—such as selenium, zinc or iron—to help them fend off disease and disabilities. However, a growing body of research into the biological role of many metal ions is now proving that Paracelsus' insight still holds true: too much of an apparently 'good' metal is toxic. Even more surprisingly, it seems that humans and other organisms might even need some 'bad' metals to function properly.

> **...a growing body of evidence shows that poisonous metals might be essential nutrients in small doses**



Although some transition metals, such as copper and zinc, are essential nutrients, others, notably mercury, are purely toxic. But the distinction between whether a metal is essential or toxic is, to some extent, only one of degree. In fact, a growing body of evidence shows that poisonous metals might be essential nutrients in small doses. For example, "cadmium has been found to be essential in a diatom, *Thalassiosira weissflogii*," noted Ute Kraemer, a plant nutrient specialist at the University of Heidelberg, Germany. This single-celled algae has a particular demand for zinc, but because this metal is deficient in the marine environment, the organism has co-opted cadmium to catalyse the conversion of CO_2 to bicarbonate instead (Lane & Morel, 2000).

Similarly, lead—historically a major contaminant in air and drinking water, and shown to hinder neuronal development, particularly in infants—might well be essential for a wide range of organisms, even if its biological role has yet to be determined. "Interestingly, lead has been shown to have a beneficial effect in animals," said Sabeeha Merchant, who investigates the biochemistry and genetics of metals metabolism at UCLA's Molecular Biology Institute (Los Angeles, CA, USA). "The molecular target of lead is not known. But lead deficiency produced anaemia and growth defects in second-generation rats, in a 1981 study."

Even arsenic, the poison of choice for many fictional murderers, is now close to qualifying as a micronutrient in animals. It seems that arsenic has a role in the metabolism of the amino acid methionine and in gene silencing (Uthus, 2003). Other work suggests that it has a positive interaction with the more important micronutrient selenium (Zeng *et al*, 2005).

In fact, if arsenic is essential for humans, its recommended daily intake would be little different from selenium, which is so important that evolution incorporated it into the rare amino acid selenocysteine—the crucial component of the antioxidizing selenoproteins that help to repair other proteins from oxidative damage. The recommended dose of selenium is 40 µg per day, whereas extrapolations from mammalian studies suggest that humans might need between 12.5 μg and 25 μg of arsenic. This is, to some extent, academic; a normal diet will contain 12–50 μg of arsenic in most parts of the world, but it shows that arsenic—the famous poison—and selenium—one of the most widely studied elements in the dietary context—could well have almost identical levels of nutritional necessity and toxicity.

Life developed a hunger for these metals very early on, perhaps as soon as the first life forms evolved. Quite simply, it was an arrangement of convenience; enzymes rely on metal ions because they were abundantly available during the early anaerobic stage of evolution. However, the later oxygenation of the atmosphere owing to photosynthesis acted to reduce the availability of some transition metals for biological use, and increased the abundance of others; put simply, the planet rusted. Iron was turned into insoluble oxides and thus life, which had already become addicted to the element as a co-factor for a wide variety of reactions, was severely challenged. Consequently, early organisms evolved elaborate means to reduce iron oxides and absorb them. By contrast, copper became more readily available and was used alongside iron in a vast array of redox reactions as part of cellular metabolism.

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Furthermore, all transition metals became toxic at quite low levels as soon as plants and cyanobacteria began to release oxygen into the atmosphere. The initial challenge for all organisms, including plants and bacteria, was to cope with the new ability of metal ions to transfer electrons in redox reactions, which represented a threat as well as an opportunity. “Each of these essential metals, such as manganese, iron, cobalt, nickel, copper, zinc, and molybdenum, became deadly with oxygen, so organisms had to develop mechanisms of chelation,” said Jonathan Gitlin, Professor of Pediatrics and Genetics at the Washington University School of Medicine (St Louis, MO, USA) and a specialist in the metabolism of metals. Organisms then release these chelated metals through controlled pathways to prevent collateral damage to proteins *en route* to their destination. “These mechanisms of chelation are so efficient that, at least for copper and most likely for all other metals, chaperones are required to rescue the metals from the chelators and distribute them for use,” Gitlin said.

These mechanisms were developed early on during the oxygen era, and they became so essential to biochemical reactions that they have remained highly conserved throughout the biota. “For example, the copper transporters (ATPases) in bacteria work in human cells, and human transporters—that cause human disease if mutated—work in plants,” said Gitlin. The importance of transition metals is also reflected in the fact that they are present in one-third of all proteins.

Even with chelating mechanisms controlling them in cells, transition metals are still dangerous at quite low doses. This leads to an important characteristic of micronutrients: a relatively low range between the recommended daily amount (RDA) and the minimum toxic level—although the actual quantities vary significantly from element to element. The typical ratio between utility and toxicity ranges between 1:10 and 1:30 according to Merchant, but can be as little as 1:5 in the case of two of the most important elements: selenium and iron. For selenium, the RDA for adults is 40 μg and the minimum toxic level is 200 μg, whereas for iron the amounts are 10 mg and 50 mg, respectively. In the case of iron, some groups have an even higher RDA, reducing the ratio still further—the RDA for pregnant women is 18 mg.

However, these tight margins are not as dangerously close as it might seem; from a nutritional perspective, for most people in wealthier nations, the RDA coincides with the levels obtained from a balanced diet. But the situation has led researchers to question not just the efficacy, but also the safety of dietary supplements, which many people have come to take almost as an insurance policy against bad health.

Historically, there has been a widespread assumption—initially shared by many nutritionists—that because micronutrients are essential, they must be safe in quite a wide range of doses and that, furthermore, there might be advantages to taking them at significantly higher levels than the RDA. The Nobel Prize-winning chemist Linus Pauling—who famously took about 50 times the recommended amount of vitamin C during his later years and claimed that he never had so much as a cold as a result—initially kindled such ideas, but this finding has now been largely discredited. According to Saverio Stranges, Associate Professor of Cardiovascular Epidemiology at Warwick Medical School in the UK, most people would be best to avoid dietary supplements if they eat a balanced diet. “There is no strong evidence supporting [the] widespread use of anti-oxidants, including multivitamins as well as trace elements,” he said.

However, nutrition is never black and white. For example, many researchers believed that supplementary selenium—if kept within safe levels—could boost resistance to a range of cancers, heart disease and type II diabetes, all of which might be triggered by oxidative stress or are associated with it. Such beliefs seemed compatible with the known role of selenium in the immune system, but defining a level at which increased selenium is beneficial has proven difficult. As Peter Hoffmann of the University of Hawaii’s Cell and Molecular Biology Department noted, “[i]ncreasing the selenium status of an individual from low to adequate certainly appears to increase most types of immunity […] However, increasing from adequate to high selenium status with supplements may increase certain types of immunity, but not others.”

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According to Stranges, there is evidence that supplementary selenium confers protection against some cancers, particularly prostate cancer, although the mechanisms involved are not yet known (Duffield-Lillico *et al*, 2002). However, a recent study found that supplementary selenium gave no extra protection against heart disease, nor did it have any significant impact on associated triggers such as platelet formation in the blood. This was contrary to many expectations, and yet the findings of another study on selenium and type II diabetes seemed even more confounding. The trial, which involved more than 1,300 people in randomized double-blind tests, found that those given supplementary selenium at the rate of 200 μg per day—close to the maximum safe level—had a slightly higher incidence of type II diabetes than those taking the placebo. "This highlights a possible detrimental effect of selenium on glucose metabolism and insulin resistance," said Stranges. There is also emerging evidence from animal models that selenium intake above the recommended level does have a deleterious effect on glucose metabolism (Satyanarayana *et al*, 2006).

> **…most people would be best to avoid extra dietary supplements if they eat a balanced diet**

As Stranges noted, these findings are significant because many dietary supplements provide levels of selenium similar to those taken in the trials, and might therefore put some people at extra risk of developing glucose metabolism complications. Yet, if the protective effect of selenium against some cancers is confirmed, there might be a case for giving supplements to people known to be at high risk, for example, men with a strong family history of prostate cancer.

Not all micronutrients are lethal in high doses, however. Some of the transition metals have a more structural role in stabilizing proteins, rather than catalysing reactions. The most striking example is the zinc finger found within many smaller proteins, including DNA transcription factors. Normally, proteins achieve sufficient strength and stability in their tertiary form through relatively weak hydrogen bonds and the hydrophobic forces between amino acids. Such bonds are adequate for larger proteins because of their cumulative effect, but not sufficient for small proteins comprising perhaps 20 or so amino acids. The evolutionary solution was to recruit a metal ion. Zinc emerged as the metal of choice, according to Merchant, for its combination of an affinity for amino-acid side chains and a lack of reactivity, which confers immunity from oxidative damage to the protein. "I guess I would say that you don't want the metal to be 'reactive' in redox reactions, like copper and iron, and therefore something like zinc is better," said Merchant.

Recent work has also shed light on the previously mysterious processes by which micronutrients are delivered safely to their targets. Copper has been most extensively studied because of its unique role in redox reactions, its flexibility and its power to catalyse many biochemical reactions. "Copper enters cells on Ctr1 [copper transporter 1] type transporters (probably these transporters also pick up silver ions in addition to the correct substrate, that is copper ions) then once it is inside, it is picked up by a 'copper chaperone'," Merchant explained. "That protein delivers copper to a transporter that pumps it into the lumen (channel) of the secretory pathway where the copper can be loaded into copper proteins that are secreted or bound to the plasma membrane."

Studying these pathways has also elucidated the interchangeability of some metals in the event of scarcity. Indeed, there are alternatives to various micronutrients that can substitute for them when necessary: copper-deficient algae, for example, substitute an iron-containing protein in place of a copper containing one (Merchant & Bogorad, 1987). "We also know that in iron-deficiency, a manganese-containing superoxide dismutase, which helps to deal with oxidative stress, can substitute for an iron-containing one," Merchant said. "There are many examples of this type of thing. It implies that there is a preference for one metal over another if the organism is provided a choice of all metals, but in a deficient environment there are backups in place."

In other cases, one metal might merely keep the seat warm for another, protecting an active binding site until the correct element comes along. Copper, for example, plays that role on behalf of molybdenum, which operates within a variety of enzymes. "During molybdenum-cofactor biogenesis, we found that copper is a placeholder protecting the highly reactive dithiolene group of molybdopterin, until molybdenum comes and is exchanged for copper," said Ralf Mendel in the Department of Plant Biology at the Braunschweig University of Technology in Germany (Mendel, 2007).

Many organisms have also evolved schemes for rationing crucial elements when essential micronutrients are scarce. Plants and cyanobacteria, for example, first allocate the available manganese to photosystem II because of its crucial role in splitting water for photosynthesis. The other main consumer of the element, the antioxidant enzyme manganese superoxide dismutase, which is found in all organisms, takes second place because plants can survive for longer without manganese superoxide dismutase activity than without photosynthesis (Allen *et al,* 2007).

In recent years, there has been an increasing focus on the differences between the needs of plants and animals in terms of micronutrients. In contrast to animals, plants have no use for arsenic and some do not require selenium. Even more strikingly, some plants can hyperaccumulate transition metals and selenium to concentrations that are orders of magnitude beyond those that would kill animals. The model plant *Arabidopsis*, for example, is able to store enormous amounts of zinc: more than 1% of its dry body mass. "Individual plant taxa have evolved hyperaccumulation of nickel, zinc, copper, manganese, selenium, cadmium, and arsenic," said Kraemer. The selective forces that allowed them to develop these mechanisms are not fully understood, but the prevailing wisdom, according to Kraemer, holds that the ability evolved as a defence against predators and invading pathogens.

> **The ability of plants to hyperaccumulate is increasingly attracting scientific interest as it has great potential for mopping up toxic metals in contaminated soils**

Selenium hyperaccumulation in the plant Prince's Plume (*Stanleya pinnata*), for example, protects it from caterpillars by acting as an immediate deterrent to feeding and by the toxic effects of selenium on the caterpillars (Freeman *et al*, 2006). However, most intriguingly, the same research showed that a variety of the diamondback moth (*Plutella xylostella*) has disarmed this defence and can eat the plant with impunity. Even more strikingly, the wasp *Diadegma insular* has, in turn, evolved to feed on the moth. Both insects have obviously developed a tolerance to extremely high levels of selenium, which shows that this ability is not confined to plants.

The ability of plants to hyperaccumulate is increasingly attracting scientific interest as it has great potential for mopping up toxic metals in contaminated soils. Possible strategies for phytoremediation could involve growing plants alongside crops to sequester toxic metals from the soil—for example, in rice-growing areas with high concentrations of arsenic in the soil. A more sophisticated and more efficient approach aims to develop transgenic plants with an even higher tolerance to metal ions to be grown on highly contaminated industrial land (Peuke & Rennenberg, 2005). Humans might thus benefit from the evolutionary arms race between plants and predators that has seen the use of micronutrients as weapons. To paraphrase Paracelsus, it is not just the dose, but also the use that determines whether something is good or bad.

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