It's a very odd thing, as odd as can be
that whatever Miss T. eats turns into Miss T.

Walter de la Mare

Chapter 11
Without bacteria there is no protein

That can't be true – I get my protein from steaks, chicken, and milk.

Yes, but where does this protein come from? Proteins come from plants, so we think of cattle grazing in lush meadows. In order to be able to synthesize proteins, plants require what is called bound nitrogen. This is nitrogen in the form of ammonia (ammonium ions), NH_3 (NH_4^+), or in form of nitrate (NO_3^-). The largest inexhaustible source of nitrogen in nature is nitrogen gas, which makes up 78 percent of our atmosphere. This nitrogen, N_2:

$$1N \equiv N1$$

is inert, not readily reacting with other elements. Neither plants nor animals are able to convert N_2 into bound nitrogen. The triple bond between the nitrogen atoms is not easy to crack. The conversion of molecular nitrogen from our atmosphere into bound nitrogen is only possible in three steps, first of all, the Haber-Bosch process based on a catalyst developed by Fritz Haber (1868–1934). It catalyzes at high temperatures (450 °C) and high pressures (300 bar) the conversion of N_2 into ammonia:

$$N_2 + 3\,H_2 \rightarrow 2\,NH_3$$

The enormous technical problems involved were solved by a team at the German chemical company BASF under the direction of Robert Bosch. The industrial Haber-Bosch process has been in use since 1913. It is the basis for the production of fertilizers and is considered one of the greatest scientific-technical achievements at the beginning of the twentieth century. In recognition of their achievements, Fritz Haber and Robert Bosch (1861–1942) were awarded the Nobel Prize.

What was going on long before that? Small amounts of N_2 can be converted into bound nitrogen by lightning in the atmosphere. The nitrogen oxides formed are further converted to yield ammonia. In prebiotic times on our planet, as mentioned before, electrical discharges in the atmosphere were quite important because they led to an accumulation of bound nitrogen. However, this source of bound nitrogen became limiting when microbial life started to flourish.

Discover the World of Microbes: Bacteria, Archaea, and Viruses, First Edition. Gerhard Gottschalk.

What lies between lightning and the Haber-Bosch process?

It is an exclusive achievement of the bacteria, and to a lesser extent of the archaea, to have "learned" to convert atmospheric nitrogen into bound nitrogen. In other words, plants and animals are not capable of using free nitrogen, N_2. The layman may think of fertilization as a process involving animal manure or plants (green manuring); in such cases, the bound nitrogen is just transferred from one organism to another. Solely the bacteria and archaea, but not all species, are able to synthesize a system of enzymes that catalyzes the Haber-Bosch reaction, but at ambient temperatures and without H_2 under high pressure. This system is called nitrogenase. It has an ingenious structure, so because of its importance it will be described somewhat in detail. The structural biologist Oliver Einsle (Freiburg, Germany) has written,

> "The reaction center of the nitrogenase is able to cleave the extraordinarily stable N_2 molecule without requiring extreme conditions as in the Haber-Bosch process. This is achieved by means of one of the most complex biological metal centers we know, by the iron-molybdenum cofactor. In the heart of this enzyme, the nitrogen molecule is bound in a highly symmetric skeleton consisting of one molybdenum atom and seven iron atoms that are interconnected by sulfur atoms. As a result, a miniaturized bioreactor is formed in which geometries, bond lengths, and angles are much more precisely modulated than would be possible in an industrial process with our current technologies. Moreover, in the case of nitrogenase, nature has implemented a nanomachine, the precision and properties of which we have not completely understood until now. The cleavage of the stable N_2 molecule is accomplished supposedly because one cleavage product, a single nitrogen atom, is bound in the center of the cofactor even more firmly than N_2 by itself. As a result, the other nitrogen atom is easily released in the form of NH_3 but, in compensation, a vast amount of energy has to be invested in order to get rid of the second nitrogen atom in the form of NH_3."

Figure 24 gives you an impression of the active center of the nitrogenase. The metals molybdenum and iron are shown along with the firmly bound nitrogen in the center as well as the iron-sulfur clusters and the carbon compounds surrounding the center. Everything is embedded in a protein structure. Another protein docks onto the nitrogenase and provides reducing power for the Haber-Bosch reaction. The nitrogenase becomes "fluffed up" with reducing power, brimming over with potential activity. However, in this state, the nitrogenase is so full of energy that it would even be able to react spontaneously with oxygen, resulting in its own inactivation. So it is absolutely essential that microorganisms shield and thus protect their nitrogenase from oxygen. Only then can the Haber-Bosch reaction be carried out. Oliver Einsle has mentioned that the N_2 molecule is held firmly within the iron-molybdenum cofactor. He goes on to say that the two N atoms are

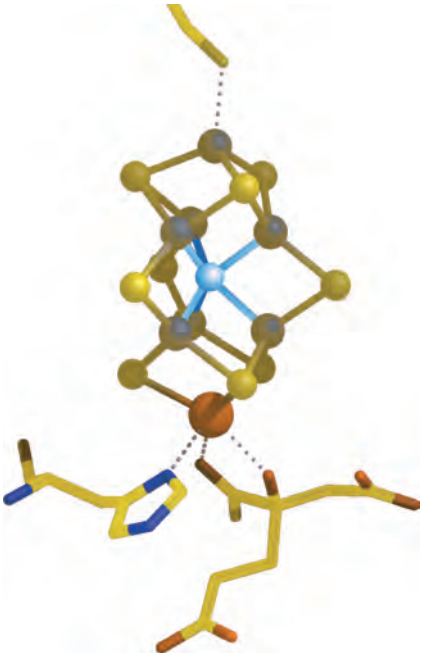

Figure 24 Active center of nitrogenase. The iron-molybdenum cofactor consists of molybdenum (orange), iron (gray), and sulfur (yellow) with the central nitrogen atom (blue). The cofactor is connected to the protein via two amino acids that affix the metal center only at its ends. In addition, an organic molecule, homocitrate, is linked to the center (bottom right). (Model: Oliver Einsle, Goettingen, Germany.)

reduced one after the other, the first one relatively easily and the second one with a high input of energy. It is helpful to imagine that one of the two N atoms is held firmly by the cofactor, like in a vise, whereas the second one flounders around and is therefore amenable to reduction. What about the *tour de force* by which the enzyme manages to extract the second N atom from the cofactor, then reduce it to NH_3 as well? This mechanism has not yet been explained.

That nitrogen fixation requires the trace element molybdenum was discovered by Hermann Bortels (1902–1979). He analyzed the composition of the catalyst used in the Haber-Bosch process. Upon noticing the presence of molybdenum, he looked whether molybdenum salts would stimulate N_2 fixation by bacteria and found this to be the case. Incidentally, there are two additional types of nitrogenase in microbes: one contains vanadium instead of molybdenum and the second one is an all-iron nitrogenase devoid of both molybdenum and vanadium. How these nitrogenases work is still unknown to scientists.

So, bound nitrogen becomes available because some microbial species are able to utilize N_2, reduce it to NH_4^+, and incorporate the latter into proteins. The biomass of these organisms serves as a nitrogen source for all other organisms on Earth. The rumen, the "first stomach" of ruminants, is a habitat in which N_2 to a large extent is bound by microorganisms. It is a nearly oxygen-free (anaerobic) environment full of reducing power. Microorganisms grow there and produce the microbial proteins, which together with plant proteins serve as a source of bound nitrogen for cattle, sheep, and goats.

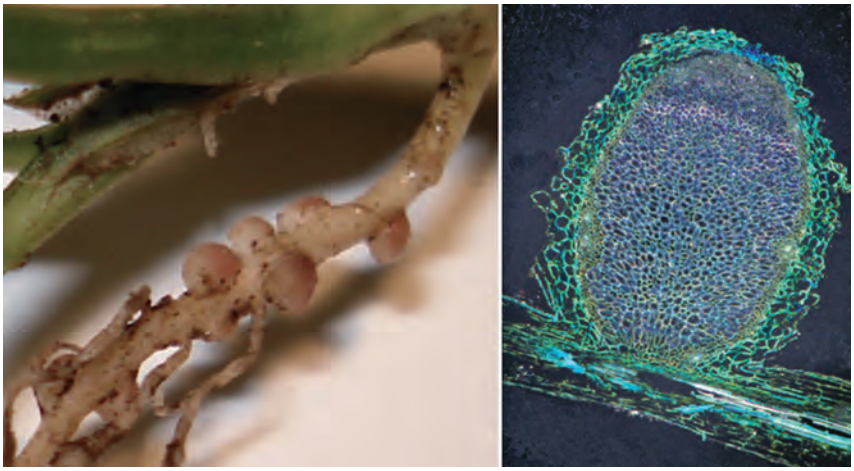

Figure 25 Symbiotic nitrogen fixation. Left: root tubercles (Christine Hallmann, Goettingen). Right: cross-section of a root tubercle filled with N_2-fixing bacteroids. (Photograph: Anne Kemmling, Goettingen, Germany.)

What about green manuring?

Not every plant species is suitable for green manuring. Those preferred are legumes such as lupines, beans, clover, and alfalfa. The rhizosphere (root area) of these plants is the site of symbiotic nitrogen fixation. This is a fascinating process. The plant, let's say alfalfa, provides at its root hairs chemical signals that are recognized by a distinct bacterial species, in this case by *Sinorhizobium meliloti*. These bacteria recognize specific plants as partners. They attach to the surface of the root hairs, which they are allowed to penetrate. Inside the root, the bacteria begin to grow and proliferate; they also induce cell division in the plant that leads to root tubercle formation (Figure 25). Something amazing then occurs in these tubercles. Under self-abandonment, the "Sinos" convert themselves into irregularly shaped cells called bacteroids. In these bacteroids, nitrogenase is formed, N_2 is reduced, and bound nitrogen in the form of amino acids is provided to the plants via their supply routes. The plants also play an important role: they embed the bacteroids in a pink substance called leghemoglobin. This substance provides just enough oxygen to the bacteroids to allow respiration but at the same time to avoid damage to the nitrogenase. Symbiotic nitrogen fixation by the legumes is an excellent example for a way in which plants put bacteria to work for production of bound nitrogen. Alfred Puehler from Bielefeld (Germany) explains the importance of symbiotic nitrogen fixation in our world by using questions and the appropriate answers:

> "Q: What is the role of symbiotic nitrogen fixation in agriculture?
>
> A: Approximately 300 kg of bound nitrogen per year and hectare (10 000 m^2 or around 2.5 acres) are introduced into an alfalfa field by symbiotic nitro-

gen fixation. Compared with the nearly 150 kg nitrogen fertilizer required for an optimal harvest of wheat per year and hectare, this figure underlines the importance of symbiotic nitrogen fixation in agriculture.

Q: Why didn't all plants acquire symbiotic nitrogen fixation?

A: The existing symbiotic systems apparently are sufficient to bring enough bound nitrogen into our ecosystem. There was no evolutionary pressure to furnish more plant species capable of symbiotic nitrogen fixation. Legumes are the pioneer plants in nitrogen-poor soils and they are followed by plants incapable of hosting nitrogen-fixing bacteria.

Q: Is it considered symbiosis when the bacteria obviously are enslaved and eventually converted into a nitrogen-fixing factory?

A: This symbiosis is profitable for the plants. They are provided with substantial amounts of bound nitrogen. As a reward, the microbial partner receives nutrients that allow it to grow in the nodules. The bacteroids, however, are no longer able to multiply, but a strong increase in the number of *Sinorhizobia* in the soil of an alfalfa field has been observed, so there is some benefit for the bacterial partner as well."

I see. Are there other plants besides legumes that serve as partners in microbial nitrogen fixation?

Of course, and I am certain that further discoveries will be made in this field. A number of woody shrubs or trees and herbs host filamentous bacteria of the genus *Frankia* that are able to fix N_2. It is interesting that some frugal pioneer plants, such as sallow thorn or oleaster, belong to this group. The seaweed *Azolla*, inhabited by cyanobacteria (*Anabaena azollae*), is also able to fix N_2. Finally, N_2-fixing bacteria live in the rhizosphere of a number of plants, for example, members of the genus *Azospirillum* in tropical grasses and species of *Azoarcus* in Kallar grass, a pioneer plant growing in salt marshes of Central Asia. Furthermore, it should be stressed that a number of microbes fix N_2 for their own purposes; in other words, the nitrogen in their proteins originates from the N_2 in the atmosphere. When they die, their bound nitrogen serves as a valuable source for other organisms. This is especially the case with cyanobacteria. They are real pioneers, for instance, on islands of volcanic origin. They are unique because they carry out photosynthesis and build up their cell material from CO_2, N_2, and minerals. N_2-fixing rice plants, however, are still a utopian dream.

It's fantastic that bacteria and archaea provide bound nitrogen, a further important prerequisite for plant and animal life.

This is still true despite the fact that our world is now overfertilized. Nearly two-thirds of the bound nitrogen still originates in the nitrogenase factories of bacteria and archaea, and the remaining third is produced in large-scale reactors of Haber-Bosch facilities.