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by

Thomas Emery
A basic objective of the Faculty Association of Utah State University, in the words of its constitution, is:

to encourage intellectual growth and development of its members by sponsoring and arranging for the publication of two annual faculty research lectures in the fields of (1) the biological and exact sciences, including engineering, called the Annual Faculty Honor Lecture in the Natural Sciences; and (2) the humanities and social sciences, including education and business administration, called the Annual Faculty Honor Lecture in the Humanities.

The administration of the University is sympathetic with these aims and shares, through the Scholarly Publications Committee, the costs of publishing and distributing these lectures.

Lecturers are chosen by a standing committee of the Faculty Association. Among the factors considered by the committee in choosing lecturers, are in the words of the constitution:

(1) creative activity in the field of the proposed lecture; (2) publication of research through recognized channels in the field of the proposed lecture; (3) outstanding teaching over an extended period of years; (4) personal influence in developing the character and social sciences, including education and business administration, called the Annual Faculty Honor Lecture in the Humanities.

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Thomas Emery was selected by the committee to deliver the Annual Faculty Honor Lecture in the Sciences. On behalf of the members of the Association we are happy to present Professor Emery’s paper.
Iron: The Problem

Iron, iron, everywhere but . . . . The plight of the ancient mariner surrounded by undrinkable water could hardly have been more frustrating than life on earth with respect to iron. Iron is the fourth most abundant element of the earth's surface, exceeded only by oxygen, silicon, and aluminum. Virtually all forms of life from the simplest bacteria to humans require iron to catalyze numerous and complex metabolic reactions. Because of the diversity and complexity of the role of iron in the life process, it has even been suggested that the origin of life on earth centered around the catalytic properties of this metal. But in spite of its abundance, the acquisition of iron for cellular needs is a formidable problem. The fact that one of every four persons in western civilization suffers from iron-deficiency anemia is testimony to the fact that the problem has not been resolved.

To understand the nature of the problem itself, we need only to review what we learned about iron in freshman chemistry. In solution, iron commonly occurs as either the ferrous form, Fe++, or the ferric form, Fe++. The ferrous form is quite soluble even at the neutral conditions of living cells. In contrast, if one neutralizes an acidic solution of the ferric ion, the iron precipitates out of solution under conditions which are still one hundred times more acidic than water itself. In fact, a simple calculation shows that in the neutral environment of living cells only one or two atoms of iron could remain in solution within the cell. When life evolved on earth, there was no oxygen in the atmosphere. Iron thus

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I am indebted to the National Institutes of Health, Grant AI 09580, for continuing research support for work on iron metabolism carried out in my laboratory, and to Liliane Emery for research assistance for more years than either of us cares to admit.
remained in the ferrous state, and in this soluble form it was plentiful in the oceans and other bodies of water. With the evolution of photosynthesis, oxygen appeared in the atmosphere. Very quickly the ferrous form of iron was oxidized to the ferric form. Geological evidence shows that vast deposits of ferric oxides were precipitated from the oceans at this time. Quickly the amount of dissolved iron decreased to an infinitesimal amount, far too small to support life. The manner in which the powerful forces of evolution allowed life to adapt to an environment virtually devoid of a soluble, utilizable form of iron will be the subject of this paper. Most of the research in this area involves microorganisms. However, a reader patient enough to follow the discussion to the end will be rewarded by learning of recent developments which may directly relate to his or her own health and well-being, facts which at the time of this writing are not widely appreciated even by practicing physicians.

With the appearance of oxygen on earth, most of the iron soon became converted to insoluble ferric silicate or ferric oxide. The red color of many soils and rock formations, particularly striking in the cliffs and arches in the Southwest of the United States, is due to ferric oxide. Given a sample of ferric oxide which he wishes to solubilize, a chemist has two choices. First, he may simply add acid. In acid, the iron quickly dissolves to give a yellow solution of the ferric ion. The second method is to add a substance called a chelating agent. A chelating agent is an organic compound which has a strong affinity for metal ions and which literally pinches the metal like a pair of pliers. In fact, the word chelate derives from the Greek word, chele, meaning the claw of a lobster. In general, chelates are very soluble in water.

Primitive living cells utilized the same method. In order to survive, they soon “learned” to synthesize relatively simple organic molecules that were effective chelating agents for iron. These substances were excreted into the environment where they effectively solubilized the metal. Because these natural iron chelates are colored bright red, we call them siderochromes, from the Greek sideros, iron, and chroma, color. In addition to being chelating agents, many siderochromes are at the same time organic acids. We have recently observed in our laboratory that during the growth of certain iron-starved microorganisms, the excretion of siderochromes may be accompanied by an increase in acid concentration one thousand times above that of the original culture. Thus, nature utilizes the same tricks as the chemist (or vice versa) to solubilize iron.
Over a billion years elapsed before the first siderochrome was discovered in 1952 by Professor J. B. Neilands at the Berkeley campus of the University of California (Neilands, 1952). I became involved in the problem several years later.

The Solution: Ferrichrome

In the fall of 1956 I was a graduate student in the Biochemistry Department at Berkeley in quest of a thesis problem. I had almost decided to work in the laboratory of Professor Arthur Pardee. He had told me of a fascinating idea he had about how single cells could control their metabolic activity, and he patiently explained to me how he might be able to prove his idea by studying the inhibition of an enzyme called aspartate transcarbamoylase. It was an exciting idea, but in the end I decided not to work with Professor Pardee for two reasons. First, I was trained as an organic chemist, and enzymes frightened me a little. Second, Professor Pardee frightened me a lot. Instead, I chose Professor J. B. Neilands as my thesis supervisor. Another student, John Gerhardt, went to work with Professor Pardee and today is an internationally renowned biochemist for his important work on the aspartate transcarbamoylase enzyme.

Several years before my arrival as a student at Berkeley, Neilands had been carrying out research in microbial iron metabolism. He had chosen to study a fungus, *Ustilago sphaerogena*, because this organism had been reported to have an exaggerated iron metabolism. When grown in a normal, iron-containing medium, *U. sphaerogena* produces up to 1% of its own weight in an iron protein, cytochrome c. Neilands observed that when the organism was deprived of iron it excreted large amounts of a new substance which turned deep red when iron was added to it. Neilands was able to isolate and crystallize small amounts of this new iron compound. Because it contained ferric ion and it was brightly colored, he named it ferrichrome. One day as I passed Neilands in the hallway of the Biochemistry building he thrust a small vial containing about twenty-five milligrams of ferrichrome into my hand and said to me, "I hear you are looking for a thesis problem. If you want a good one, figure out the chemical structure of this stuff." I decided to accept the challenge, not knowing that another student had spent nearly two years on the problem and left in discouragement.
After eighteen months of hard work, I had accumulated five large notebooks of experimental data, but I knew no more about the structure of ferrichrome than did the previous student. Neilands had just left for a year's sabbatical leave in Sweden and England, and I was very discouraged. Ferrichrome is a relatively small molecule, and it is noted for holding onto the iron extremely tightly. My chemical analyses had shown that, in addition to one iron atom, ferrichrome contained three molecules of acetic acid, three molecules of the amino acid, glycine, and some ornithine, an amino acid which is rather rare in biological molecules. The amount of ornithine plagued me. Some days my analysis would yield 1.5 molecules of ornithine, some days almost none at all. At that time, one of the world's few functional amino acid analysis machines was located in the laboratory of Dr. Heinz Fraenkel-Conrat up in the virus area on the fifth floor, an area forbidden to graduate students. The machine was being used to decipher the structure of the coat protein of tobacco mosaic virus, work which would later bring fame to Dr. Fraenkel-Conrat. The machine was operated by a postdoctoral assistant to Dr. Fraenkel-Conrat, a gentle Japanese fellow named Dr. Tsugita. One day at 2 o'clock in the morning I sneaked up on the fifth floor and handed Dr. Tsugita a hydrolysate of ferrichrome, explaining to him that I would be forever indebted if he would be willing to run my sample through the amino acid analyzer. After making me promise that Professor Fraenkel-Conrat would never know, he agreed to do it for me. (Fraenkel-Conrat still does not know. I assume that he will not read this essay.) I shall never forget my excitement the next day when Dr. Tsugita came running up to me with his yards of paper recordings taken from the machine. Of course, the three molecules of glycine clearly showed up, but how much ornithine, I asked. None at all was the answer, but instead Dr. Tsugita showed me a peak which agreed with no known amino acid. Off to Val's Pizza Parlor to celebrate our discovery of a new amino acid! Dr. Tsugita made me swear that I would not mention his name in my thesis for fear that Professor Fraenkel-Conrat would throw him out of the lab for using the analyzer for something other than tobacco mosaic virus.

One month later, after many long hours of experimentation and much sweat, I was able to firmly establish the structure of the new amino acid as a derivative of ornithine, 8-N-hydroxyornithine. It is the
amino acid ornithine in which one of the nitrogen atoms has been substituted with an –OH group:

\[
\begin{align*}
\text{ornithine} & : \quad \text{H}_2\text{N}-(\text{CH}_2)_3-\text{CH}-\text{COOH} \\
\delta\text{-N-hydroxyornithine} & : \quad \text{HO-NH}-(\text{CH}_2)_3-\text{CH}-\text{COOH}
\end{align*}
\]

This compound had never before been reported in the literature, and only a few other examples of hydroxylamino acids were known. I realized that the reason for the previous ambiguous analyses was that traces of iron would cause variable amounts of N-hydroxyornithine to be converted to ornithine during acid hydrolysis of ferrichrome. I was able to put this fact to use in order to determine how much N-hydroxyornithine was actually present in the ferrichrome molecule. By carrying out the hydrolysis with hydriodic acid instead of hydrochloric acid, the –NOH group was quantitatively reduced to an –NH₂ group, yielding ornithine cleanly. Exactly three molecules were found, proving that ferrichrome itself contained three molecules of N-hydroxyornithine. Armed with this information, I could account for all of the parts of the ferrichrome molecule and correctly draw its complete structure:

![Figure 1. The structure of ferrichrome.](image)

Consequently, instead of writing a monstrous thesis describing unsuccessful experiments on the ferrichrome molecule, I was able to write the
shortest Ph.D. thesis in the department, entitled simply "The Structure of Ferrichrome." One picture is worth 10,000 words to an organic chemist!

Our first publication on the structure of ferrichrome appeared in the Journal of the American Chemical Society in 1961 (Emery and Neilands, 1961). As so often occurs in science, within a few months the structure of another siderochrome was published. A group in Switzerland, unknown to our laboratory at that time, had independently isolated an iron-chelating agent, which they called deferrioxamine, and they had determined its chemical structure (Keller-Schierlein and Prelog, 1961). Since that time, several dozen similar compounds have been discovered. In fact, it appears to be a general rule that if a microorganism is starved for iron, it will excrete a siderochrome into the environment to sequester that metal (Emery, 1978). It is interesting that microbiologists had previously overlooked this almost universal phenomenon despite the fact that several grams of siderochrome are frequently produced in each liter of culture medium.

Examining the structure of ferrichrome or similar siderochrome indicates that the Fe³⁺ is surrounded by six oxygen atoms located at the six corners of a regular octahedron. This is the type of chelation chemistry that Fe³⁺ prefers. The groups which supply these oxygens are called hydroxamic acids, -CO-NHOH. There are three of these in the ferrichrome molecule, each supplying two of the six oxygens that surround the metal. When several chelating groups are found on the same molecule, a phenomenon called the "chelate effect" is produced, making the affinity for the metal much greater than if the groups were on individual molecules. Thus, ferrichrome binds Fe³⁺ approximately a million times more strongly than simple hydroxamic acids. Although organic chemists have known for almost three quarters of a century that hydroxamic acids form strong complexes with iron, no one had ever thought of putting three such groups on the same molecule to give a super iron-binding substance. It is testimony to the power of evolutionary forces that this mechanism was initially utilized by simple microorganisms over a billion years ago. It also provides me with an excuse as to why I did not discover the structure of ferrichrome sooner. No compound, either naturally occurring or synthetic, containing three hydroxamic acid groups was known prior to the discovery of ferrichrome.
The Role of Ferrichrome as Nature's Gcritol

How does ferrichrome work to solve the cell's problem of iron assimilation? When faced with iron starvation, a complex control system alerts the cell to impending disaster. Although we still do not understand the molecular details, we do know that cellular protein synthesis is diminished and cellular pools of amino acids are instead diverted to the synthesis of deferriferrichrome, i.e., the organic part of the ferrichrome molecule lacking the iron. It is as if the cells realize that further synthesis of proteins will be useless without iron. The deferriferrichrome is excreted from the cell where it solubilizes iron in the environment. The iron-binding properties of this substance are so great that in the laboratory iron is actually pulled out of Pyrex glassware or stainless steel! In nature, oxides of iron are readily solubilized. When the \( \text{Fe}^{+++} \) is chelated, each of the three hydroxamic acid groups loses a proton, \( \text{H}^+ \), to leave three negative charges on the molecule. These three negative charges balance the three positive charges of the \( \text{Fe}^{+++} \) to give an electrically neutral molecule. This is important because charged molecules have a difficult time traversing cellular membranes. In addition, as the ferrichrome wraps itself about the iron a dramatic change in shape, called a conformational change, occurs. The cell can therefore readily distinguish between the iron-free molecule, which is excreted, and the iron chelate, which is taken up by the cells.

In 1957, my professor, J. B. Neilands, suggested that ferrichrome acts as a transport agent to get iron into the cells (Neilands, 1957). It was not until 1971 that work in my laboratory provided definite evidence that his suggestion was correct. The organism that produces ferrichrome, *Ustilago sphaerogena*, possesses a specific transport system which virtually sucks ferrichrome up from the surroundings, thus concentrating iron within the cells (Emery, 1971). It is generally believed now that no metal ion can freely enter the cell. Specific chelating agents are probably produced and act in a manner similar to ferrichrome to sequester other essential metals, such as magnesium and zinc. Chelates that carry metal ions into the cells are called ionophores, literally meaning "to carry ions." Ferrichrome can be considered to be a ferric ionophore. The general term for an iron transport substance is siderophore, "to carry iron." Ferrichrome was the first metal transport
system to be examined, and it has been intensively studied during the past decade.

Before leaving this discussion of iron transport, one final point must be clarified. Cells utilizing a siderophore transport system have seemingly painted themselves into a corner. They have succeeded in accumulating iron, but it is in an unavailable form. Siderophores bind iron so tenaciously that no other cellular substance is capable of removing it. The solution to this problem is simple and is based on the principle that hydroxamic acids bind ferric, Fe\(^{+++}\), very strongly but they have virtually no affinity for ferrous ion, Fe\(^{++}\). Thus, the cell merely has to reduce the metal and it will be released from the chelate. Numerous workers have shown that cells contain specific enzymes, called siderophore reductases, which catalyze this reaction. Siderophores bind Fe\(^{+++}\) billions of times more strongly than Fe\(^{++}\), and no other known substance shows this extreme preference for the oxidized form of iron. Once the iron has been removed, the organic portion of the molecule can be excreted again to pick up some more iron. Ferrichrome thus acts as a “bucket brigade,” shuttling in and out of the cell carrying iron rather than water.

**Who Cares?**

Each month Senator William Proxmire awards to some scientist what he calls the “golden fleece award.” The award is made to the scientist who, in Senator Proxmire’s opinion, has been given federal funds to carry out a stupid research project. Over the past fifteen years, millions of dollars of federal funds have been awarded to scientists around the world in order to carry out basic research on iron metabolism in some of the weirdest microorganisms imaginable. How can this be justified? Out of fear that I may be the next recipient of the golden fleece award, I would like to devote the remainder of this essay to discuss some of the ways this research has led to practical benefits in terms of human health.

**Iron Overload—Too Much of a Good Thing**

Everyone is familiar with the problem of iron-deficiency anemia, a problem in which too little iron is absorbed from the intestines into the blood stream. The opposite problem, iron overload, is less well known because it is much rarer. On the other hand, it is usually much more serious. Unlike iron-deficiency anemia, iron overload is often fatal.
There are basically two reasons why iron overload may occur. The first is due to a rare disease, called haemochromatosis (Dreyfus and Schapira, 1960). For unknown reasons, people with this affliction absorb too much iron. Most normal adults absorb about one milligram of iron per day from the diet (menstruating women need about twice this amount because of monthly blood losses). In order to maintain iron balance, we must also excrete one milligram of iron per day. Amazingly, humans do not possess a metabolic mechanism to handle iron excretion. Iron is apparently the only dietary element for which this is true. It is as if Nature has had such a difficult time assuring the adequate assimilation of iron that she decided that adequate iron excretion would never be a problem. Fortunately for most of us, the average amount of iron lost haphazardly each day almost exactly balances the amount we absorb.

Individuals suffering from haemochromatosis absorb about two milligrams of iron per day, instead of one. Although this tiny amount of iron does not even cover the head of a pin, it amounts to prodigious quantities over the years. A normal individual contains three to four grams total body iron. By the age of forty, an individual with haemochromatosis may have accumulated twenty or thirty grams of excess iron. The excess iron is found in many tissues, such as the liver. Since the tissue cannot hold such large amounts of iron, the metal actually deposits and death often ensues. The abnormal iron deposits are actually visible in the liver, and the disease is sometimes referred to as the "rusty liver disease."

Haemochromatosis is a rare genetic disease, and most physicians probably never encounter a case. However, there is another form of the affliction which is far more common. It is called secondary haemochromatosis. In this case, diet plays an important role. Certain diets greatly increase the amount of iron absorption. Excessive alcohol is an example (Geritol is 12% alcohol). Cirrhosis of the liver is common in alcoholics. Although alcohol is almost certainly the primary factor, some investigators believe that the problem is exacerbated by increased iron absorption. Secondary haemochromatosis is common in VA hospitals, where alcoholism is often encountered. Some people believe that the ruddy complexion (and high incidence of liver problems) of people living in southern France is not so much due to the sunny climate as it is to the high iron content of southern French wines — as much as sixteen milligrams per liter as compared to less than three milligrams per liter.
for American wines. The most extreme example of secondary haemochromatosis occurs in the Bantus of South Africa, who brew a beer in iron kettles. This beer, called kafir, contains over forty milligrams per liter, and it is not uncommon for an average Bantu male to consume over 100 milligrams of iron each day in the form of beer alone. Secondary haemochromatosis is a common form of death for Bantu males (Bothwell, 1960).

Although primary haemochromatosis cannot be cured, it is obvious that the problem might be alleviated if the body could maintain an appropriate iron level. One way of doing this, still commonly practiced, is bloodletting. Another possibility is to administer a chelating agent, which would grab onto the metal and remove it from the tissues so that it might be excreted in a harmless form in the urine. Unfortunately, most chelating agents are not specific for one particular metal. A chelating agent such as ethylenediaminetetraacetic acid would not only effectively remove iron, but also other essential metals, such as calcium and zinc. As discussed above, however, siderophores produced by microorganisms are highly specific for Fe^{++}, which is the form of the iron deposited in the body. Vladimir Prelog, who was the leader of the Swiss group which isolated deferrioxamine in 1961 and is a recent winner of the Nobel Prize in chemistry, was the first individual to realize that this property of deferrioxamine might be useful in treating haemochromatosis. In collaboration with a haematologist, he initiated treatment on a seventeen-year-old girl dying of primary haemochromatosis. The treatment consisted of infusing over one gram per day of deferrioxamine into the patient. The results were dramatic (see Figure 2). Although the normal excretion of iron in the urine is less than one milligram per day, immediately after starting the treatment over fifteen milligrams of iron appeared in the urine and reached a maximum of almost forty milligrams on the tenth day. Similar results were obtained with other patients. Because of the red color of the ferrioxamine chelate, the urine of patients during treatment resembled port wine, which was very alarming to nurses not alerted to the situation!

Soon after the discovery of this medical use of siderophores, the Ciba-Geigy pharmaceutical company started growing large quantities of the microorganism and sending deferrioxamine to hospitals around the world in fifty-pound sacks. This was most impressive to me, having accomplished my thesis research on just a few hundred milligrams of
ferrichrome. Deferrioxamine is now packaged under the trade name of Desferal. Unfortunately, however, continued infusions of large amounts of Desferal over long periods of time lead to undesirable side effects, which is why bloodletting is still performed on haemochromatosis patients. In the United States, the National Institutes of Health is sponsoring ongoing research for the discovery or synthesis of new siderophores which will not exhibit deleterious side effects. Why not synthesize siderophores in the laboratory instead of being dependent upon those supplied by microorganisms? In fact, both the Swiss and the Japanese have reported the chemical synthesis of ferrichrome. However, the synthesis is tedious and the yields are very poor. The problem lies with the instability of one of the constituents of ferrichrome, namely, N-hydroxyornithine.

A colleague, Dr. Richard Olsen, and I have recently been awarded a National Institutes of Health grant to attempt a novel chemical synthesis of siderophores. You will recall that the iron is held in ferrichrome
by three hydroxamic acid groups, \(-\text{CO-NOH}\). If one imagines the hydroxamic acid groups to be excised from the molecule and turned around, the following structural change would occur:

![Diagram of chemical structures](image)

**Figure 3. Chemical comparison of ferrichrome and side-chain retroferrichrome.**

We call this new compound side-chain retroferrichrome. Inspection of molecular models indicates that this change would have little or no effect on the iron-binding center. Side-chain retroferrichrome should bind iron as well as ferrichrome itself. Although the two structures may appear similar, they are dramatically different to the synthetic organic chemist. Whereas in ferrichrome the \(-\text{N-OH}\) group is supplied by N-hydroxyornithine, in the new compound it is supplied by methylhydroxylamine, \(\text{CH}_3\text{NHOH}\), a substance that can be bought in pound quantities. The amino acid attached to the ring system is no longer the rare, unstable, N-hydroxyornithine, but rather a substance called \(\alpha\)-aminoadipic acid, which is also readily available commercially. Thus, side-chain retroferrichrome should be relatively easy to synthesize in the laboratory. We should also be able to make alterations in the molecule during chemical synthesis, and thus build into the molecule chemical and physical properties, such as greater or lesser water solubility, that might be medically advantageous. Availability of a series of synthetic derivatives could lead to the discovery of a substance that would not only be more effective in removing iron from the body, but could also be administered orally. In a recent symposium on iron metabolism, several participants
agreed, “There is consequently a serious need to develop iron chelating agents which can be used to combat iron overload. Unfortunately, those in common use today are not very satisfactory; they are by no means as effective as could be hoped and oral administration is not currently possible due to poor absorption” (May et al., 1978). We hope that our research may alter this situation, and at the same time avoid our becoming recipients of Senator Proxmire’s golden fleece award.

Iron Poisoning

Every year many small children around the world die of iron poisoning. It has been estimated that 10% of all childhood deaths from poisoning may be attributed to iron. We may ask two questions. First, why is a substance so essential to good health also poisonous? Second, why is iron poisoning so common? We do not know the answer to the first question. For unknown reasons, large amounts of iron cause massive tissue destruction in the gastrointestinal tract. Ingestion of large amounts of iron salts causes vomiting, collapse of the vascular system, and often death. The answer to the second question is simple — ignorance on the part of the parents. It is common medical practice for physicians to prescribe iron supplements for women during the third trimester of pregnancy. This is necessary to insure that the newborn will be supplied with an adequate amount of iron during the first few months of life. Consequently, bottles containing several grams of iron are often found within easy reach on the kitchen or bathroom shelf. It has always seemed incredible to me that aspirin is packaged in bottles requiring the intelligence of Einstein and the strength of a gorilla to open, while bottles containing iron supplements can be opened easily with one hand. I also consider it an obscenity that the manufacturers of these supplements place the iron in candy-coated tablets, which closely resemble M & M candy. Thus, the small child supposedly sneaking a handful of candy actually may swallow several grams of iron. It is indeed tragic that by attempting to guarantee the health of her unborn child, a woman may carelessly cause the death of another child in the house.

Several cases of childhood iron poisoning, and at least one case of adult iron poisoning, have occurred in the Cache Valley area within the past few years. Much to my dismay, one example is the child of a friend of mine in Smithfield. Fortunately, to the best of my knowledge, the
individuals are still alive—thanks to microbial iron metabolism. Desferal has found clinical use not only in cases of chronic iron overload, but also in cases of acute iron poisoning. Toxicology centers and hospital pharmacies now usually have Desferal on hand. Large quantities of this natural iron-chelating agent are quickly administered to the patient. The Desferal travels throughout the body and rapidly binds the unwanted metal. The harmless Desferal-iron chelate, ferrioxamine, is excreted in the urine.

Iron and Infectious Disease

I would now like to turn to an area of human iron metabolism that has only recently received attention and is still somewhat controversial. This aspect of iron metabolism is not discussed in medical school curricula and is not generally appreciated by most practicing physicians. As previously mentioned, virtually all living cells require iron for growth and survival. One way to prevent bacteria from growing, and thus to prevent an infection, is to deprive them of iron. Nature apparently discovered this obvious fact a long time ago. Egg whites contain an interesting protein called conalbumin. Conalbumin, sometimes called ovotransferrin, is very interesting in that it tightly forms a chelate with Fe++. In fact, it is one of the few natural products which has an affinity for Fe+++ comparable to microbial siderophores. Unlike siderophores, the iron is not held by hydroxamic acid groups, but rather by specific amino acid residues that surround the metal. Is this property of conalbumin merely fortuitous, or is there a reason? Most researchers in iron metabolism believe the latter. Eggs are an excellent incubation medium for bacterial growth. And eggs crack. Eggs are not usually located in the most sterile of environments, and a cracked egg is vulnerable to bacterial infection. If this happened frequently, the species could not survive. We believe that nature supplied the egg with conalbumin to protect it from infection. All the available iron is chelated by the protein, and it is thus unavailable for bacterial growth. This is readily observed in the laboratory. A small amount of conalbumin added to a bacterial culture prevents growth. We can only speculate as to how important this phenomenon is in nature. However, a former student of Professor Neilands, John Garibaldi, made an interesting observation several years ago (Garibaldi, 1962). At that time there was a severe problem with egg spoilage in certain regions of central California.
Dr. Garibaldi reasoned that if the well water used by ranchers to wash eggs contained a high concentration of iron, the conalbumin might become saturated with the metal. Once the capacity of conalbumin was exceeded, the excess iron would be available for bacterial growth, leading to spoilage. Dr. Garibaldi tested this hypothesis by analyzing the well water of different chicken ranches for iron content. He found a direct correlation between iron content and the amount of egg spoilage. Might such a mechanism operate in animals, including humans? The answer is probably yes. Certain parts of the body are especially vulnerable to infection. The eyes are an example. The mucous membranes of the eye are constantly exposed to airborne bacterial infection. Dr. Alexander Fleming, the discoverer of penicillin, observed long ago that tears contained an enzyme, lysozyme, which catalyzes the destruction of bacteria by breaking apart their cell walls. The reader may be interested to know that the commercial source of lysozyme today is not tears, but egg white. If eggs contain both lysozyme and conalbumin for protection, might not the eyes also? While I was still a student in Neilands’s lab, I considered this possibility and carried out some preliminary experiments. Unfortunately, I could reach no conclusion. More recently, however, other scientists have indeed found that tears contain a protein that is extremely similar to conalbumin. It is called lactoferrin, and it binds iron almost exactly as strongly as conalbumin (Hoffer et al., 1977). Because lactoferrin is not known to play any other biological role than binding iron, it seems logical to conclude that its role in the eye is to inhibit bacterial infection in the same manner that conalbumin protects eggs.

Too Much of a Good Thing

If a scarcity of available iron prevents bacterial growth, might not an individual’s susceptibility to infectious diseases be closely related to his or her iron nutrition? Although there is no evidence at present that eye infections are related to iron abundance, there is an increasing body of medical literature that such a relationship does exist in blood.

When most of us think about iron in humans, we rightly think of blood. About 75% of all of our iron occurs in the blood, and most people know that the red color of blood is due to the iron-containing protein, hemoglobin. You would hardly think that a bacterium entering the blood through a cut or a scratch would have to worry about a
sarcity of iron. But this is exactly what occurs. The iron is packaged inside of the red blood cell and is not accessible to the bacterium. In fact, it is not even accessible to the other cells of the body. Nevertheless, our blood is responsible for transporting iron from the intestinal tract, where it is absorbed, to all other tissues. This is not the role of hemoglobin, however, but rather this job is delegated to a protein called transferrin, occurring in the plasma rather than in the red blood cell. Transferrin is a protein perfectly designed to bind Fe$$^{+++}$$ strongly, and it may come as no surprise at this time to learn that transferrin bears an amazing resemblance to lactoferrin and conalbumin.

For all practical purposes, the only available source of iron for a microorganism finding itself in the bloodstream is the iron bound by transferrin. But transferrin binds iron with an extraordinary tenacity. How can the microorganism remove it? Simply by doing what it does under our laboratory conditions of iron deprivation, that is, by excreting a siderophore into the blood plasma. The binding strength of siderophores and transferrin for Fe$$^{+++}$$ is almost identical, so a competition begins between your own transferrin and the microbial siderophore. If the siderophore wins, you are faced with an infection. This relationship between the iron nutritional status of an individual and the susceptibility to infectious disease has been discussed in detail by E. D. Weinberg (1978). I would like to cite a specific example.

About ten years ago it was common practice in certain New Zealand hospitals to give iron supplements to infants soon after birth (Barry and Reeve, 1973). This practice was stopped when it was found that the incidence of certain bacterial infections was eight times higher in the treated infants than in infants who were left alone. Thus, in a well-intentioned effort to make children "stronger" by increasing their iron levels, the doctors were actually increasing the chances that children would be exposed to potentially fatal infections. According to Sussman, "Iron plays an important part in determining virulence and possibly even pathogenicity in experimental infection" (Sussman, 1974). Weinberg has called this phenomenon "nutritional immunity."

According to the concept of nutritional immunity, an animal may protect itself against certain infections by maintaining its iron nutritional status at a lower level than is commonly thought to be ideal for good health. A rosy complexion has always been regarded as a sign of health. Within the past few years, however, a large body of medical research
is beginning to suggest that borderline iron-deficiency anemia may not always be as bad as it appears at face value. It has been known for a long time that at the onset of some infections, iron absorption from the intestines rapidly decreases. This observation is easy to interpret if one accepts the concept of nutritional immunity, i.e., the body is making every effort to deprive invading microorganisms of iron to delay their growth so that our immune system can destroy them. A human will not die if deprived of iron for days, or even weeks. Rapidly growing bacteria cannot fare so well without iron. As previously mentioned, because of menstrual iron losses women have greater demands for dietary iron than men. Women tend to suffer from iron-deficiency anemia much more than men. Is it not possible that women's greater resistance to infection is related to their lower levels of body iron?

Babies and Iron Nutrition

Except for the immediate threat of starvation, infection poses the greatest hazard for the survival of the newborn child. Like eggs, the infant is constantly exposed to bacteria. It is not surprising that nature has evolved numerous devices to protect the infant; without such protection, it is likely that none of us would be here. In this section I would like to briefly discuss infant survival with respect to iron nutritional status. It is generally agreed that human milk is the ideal food for the newborn. Beverly Winikoff has written a fascinating article relating breast feeding to nutrition, population, and health (Winikoff, 1978). Breast milk not only contains a near ideal combination of easily digestible foods, but also an array of substances designed to immunize the child against infection. Surely iron, the indispensable element for life, has not been overlooked? An article clipped from the Logan Herald Journal of October 15, 1978, describes the concern of doctors about iron malnutrition in infants. The article states that "iron must be replaced through iron from breast milk . . . ." This is nonsense! Cow's milk contains less than one milligram of iron per quart, and human milk contains even less. To make matters even worse, milk contains the iron-binding protein lactoferrin. When lactoferrin binds iron, it changes its structure and becomes extremely difficult to be digested by intestinal enzymes. Nature has seemingly made every effort to make iron unavailable to the newborn child. How could she have erred so badly?
Iron experts believe that Nature has not erred at all, but rather that iron is purposely kept from the diet in order to protect the child from infection. Unlike the well-intentioned New Zealand doctors mentioned above, supplemental iron is actually kept away from the infant. Babies are born with more than enough iron to take care of their needs for months—just how many months is a matter of controversy. A great deal more research is needed to decide when, or if, supplemental iron should be added to the diet.

There is an interesting sidelight to the low levels of iron in the diet of breast-fed infants and the development of the child's digestive system. As far as we know, iron is required by all forms of life with one exception. The exception is the lactic acid bacteria. No one has yet been able to demonstrate an iron requirement for these organisms. These bacteria are found in the digestive tract of young animals, and they digest lactose, or milk sugar, so that the digestion products are absorbable by the intestine. Because of the virtual absence of iron in milk and the strong binding of what little iron there is by the lactoferrin protein, it is obvious that a microorganism could thrive in an infant's intestinal tract only if it had a negligible requirement for iron.

**Iron and Fever**

Warm-blooded animals have an elaborate physiological control system to maintain a constant body temperature. In man, this temperature is about 37°C. Any significant deviation from this temperature puts stress on the body and makes it difficult to maintain metabolic processes at their normal rates. Why then, during sickness, should the temperature rise? It would seem that development of fever would cause things to go from bad to worse, and make it more difficult for the body to recuperate. Fever is a complex and little understood phenomenon. Nevertheless, it is becoming more and more evident that fever can, in fact, be very beneficial to an organism. It is common practice today to make every effort to reduce fever during illness. It would not surprise me at all if in the future we are told to let a fever run its course because of its benefit to the recuperative processes. I would like to discuss one aspect of fever as it relates to iron metabolism and infectious disease.

Professor Matthew Kluger has recently written an article on the evolution and adaptive value of fever (Kluger, 1978). He cites an
interesting study by Vaughn, who investigated the survival of desert lizards, iguanas, after infection with a pathogenic bacterium. Reptiles cannot regulate their body temperature. When placed in a chamber that is held at 50°C at one end and 30°C at the other end, they will move back and forth to try to adjust their bodies to a preferred temperature of 38°C. However, after infection, they spent more time at the hot end of the chamber, purposely raising their body temperature. If they were not allowed to do this, their survival rate decreased. Below is a graph of a similar study by Kluger and collaborators who infected iguanas with bacteria and then kept groups of the infected lizards at different temperatures.

**Figure 4.** Survival rate of desert iguanas injected with a pathogenic bacterium, *A. hydrophila*, and then maintained at various temperatures. Numbers in parentheses are total number of animals in test group. From Kluger (1978).
The graph clearly shows that lizards held at "fever" temperature of 42°C survived quite well — much better than the animals held at their good health-preferred temperature of 38°C. The article goes on to cite similar studies with warm-blooded animals.

Although the protective value of fever during infection must certainly be very complicated, I would like to point out one aspect which is related to iron. In the course of his studies on microbial siderophore biosynthesis, Garibaldi made an interesting observation (Garibaldi, 1972). If the temperature of cultures was raised a few degrees above the normal growth temperature of 37°C, the organisms would survive quite well, but they ceased to produce siderophores. Garibaldi was the first to point out the possible medical significance of this observation. Without iron, the bacteria cannot grow within the body. We have seen that during infection the body restricts the levels of blood plasma iron in order to make it more difficult for the microbes to grow. It may be possible that one of the reasons that fever is produced is to inhibit the ability of the microorganism to produce siderophores, which might otherwise effectively compete for the last traces of iron in the blood plasma. Thus, normal body temperature and adequate iron nutrition may be just fine for people in good health, but may actually be detrimental at the onset of infection.

Over 300 years ago, Sydenham said that "fever is a mighty engine which Nature brings into the world for the conquest of her enemies." Perhaps loss of appetite to restrict iron intake is one of the pistons of that engine. A popular old wives' tale is to "feed a cold but starve a fever." Fevers may be caused by bacterial infection, the infection being slowed by lack of iron. Colds are caused by viruses. Viruses do not contain iron!

Iron and Plants

It would be amiss for a professor at a land grant university, whose students are called Aggies, to conclude a discussion of iron without a word about the role of this element in agriculture. Just as in animals, many of the enzymes essential for plant life require iron. Perhaps most important, the biosynthesis of the green pigment, chlorophyll, also requires iron. Lack of iron leads to diminished chlorophyll synthesis and a yellowing of the plant. Such a condition is called chlorosis. Chlorosis is
common in alkaline soils, such as are often found in Utah, because of the extreme insolubility of oxides of iron in alkaline solution. Without sufficient chlorophyll, plants cannot carry out photosynthesis, and greatly diminished crop productivity results. Farmers and horticulturists have long recognized this problem and have made strenuous efforts to solve it. Many years ago children in Hawaii earned pocket money by taking iron turnings from foundry lathes and placing one turning on the top of each pineapple plant in the plantations. The acidity of these plants is apparently sufficient to solubilize metallic iron. Today, synthetic iron chelates are used. The spraying of many tons of commercial iron chelates on agricultural crops, as well as on residential lawns, is a multimillion dollar industry.

Most commercial iron chelates used for agricultural purposes are derivatives of a substance called ethylenediaminetetraacetate, or EDTA. EDTA forms a very stable chelate with Fe^{3+}, and it is surprising that plants are able to remove the metal from this foreign compound. In order to do this, they must completely degrade the EDTA itself by metabolic processes. The final products of EDTA degradation are not known, but it is interesting that EDTA contains organic amines, which in some cases are known to be metabolically converted to cancer-producing compounds. When one considers the many tons of EDTA sprayed on crops each year, it would appear that this is one area thus far overlooked for criticism by environmentalists.

Quite obviously, plants were around long before EDTA was invented. How do plants cope with iron deficiency in nature? There is no evidence that higher plants mimic microorganisms and excrete siderophores. However, they often excrete from the root system a complex mixture of organic acids. This mixture is generally called "humic acid." These acids apparently play the same role in plant iron metabolism that siderophores do in microorganisms. The soil becomes more acidic, thus increasing the solubility of iron. Furthermore, many of the acids, such as citric acid, are respectable chelators of iron, although not in the siderophore class. Thus, plants make use of the same two principles for obtaining iron—acidity and chelation. Sometimes, this may actually work to the disadvantage of the plant. If the soil under the plant is porous, the solubilized iron may actually drain away from the root system. Epstein gives a dramatic example of this in the Kauri pine, *Agathis australis*, in New Zealand (Epstein, 1972). The tree is growing
on soil rich in oxides of iron, but the humic acids excreted by the tree have leached the iron away from under the tree, leaving a base of pure white silica behind.

Although higher plants do not synthesize siderophores, I believe that siderophores may play an important role in plant iron nutrition. Species of the fungus *fusaria* are commonly found growing on plant roots. About fifteen years ago we found that this fungus excretes a powerful iron-binding siderophore, fusarincine C, when grown under iron deprivation. In laboratory experiments, we subsequently found that fusarincine C could be taken up by the root system of soybeans and tomatoes, and the iron was distributed to the leaves. Although growth of this fungus on plant roots is considered to be a pathological condition for the plant, it may be that under certain conditions a symbiotic relationship occurs wherein the soil microorganisms provide siderophores which serve to supply iron to the plants. This possibility has never been seriously investigated, although we have shown that siderophores are always found in randomly collected soil samples. If it were not for economic considerations, the use of siderophores rather than EDTA in agricultural applications would be far more ecologically sound.

Another observation that suggests that microbial siderophores may play an important role in iron nutrition in plants was reported by Neilands in an article entitled, "Ferrichrome Mimics the Green Island Effect" (Atkin and Neilands, 1972). It is sometimes observed that leaves yellow with chlorosis have small patches of green, which are called "green islands." They sometimes occur at the site of virus infection. Neilands made the interesting observation that green islands could be produced on leaves by the application of trace amounts of ferrichrome. The effect is specific for ferrichrome and can be produced by amounts as small as ten nanograms! These results strongly imply that ferrichrome is specifically supplying catalytic amounts of iron to allow chlorophyll synthesis to proceed in an otherwise defective leaf.

Another line of evidence implicating siderophores in plant iron metabolism relates to copper. Plant scientists have long known that the presence of high concentrations of copper in the soil antagonizes the assimilation of iron, which leads to chlorosis. Copper is chemically very different from iron, and there is no obvious biochemical relationship between these two metals to suggest an explanation for the copper effect. However, it is known to chemists that hydroxamic acids—the
fundamental chelating groups of many siderophores—chelate with copper to form an insoluble blue precipitate. I observed many years ago that if the iron is removed from ferrichrome and copper is added to the colorless solution, a blue precipitate quickly forms. I would suggest, therefore, that when soil microorganisms excrete iron-free siderophores in their attempt to sequester iron from the soil, the copper-siderophore complexes precipitate and become unavailable for iron metabolism for both the microorganism and the plant. Although iron is bound to siderophore ligands much more strongly than copper, the latter metal is much more soluble, and the precipitation occurs before the iron has a chance to be solubilized. Once the copper precipitate forms, it would be very difficult for iron to displace copper from the insoluble copper chelate. No one has ever examined soils in copper-rich regions to see if copper-siderophore chelates are present.

Conclusion

In spite of many years of intensive research, some of the most fundamental problems of human iron metabolism remain unsolved, for example, the mechanisms by which iron is absorbed from the intestine and later released to the tissues according to need. Because of the relative simplicity of microbial systems, advances have come more quickly in this area. The detailed mechanism of iron transport in microbes is now quite well understood, and hopefully this understanding will be of value to future researchers in the medical sciences.

I have described in this essay some of the ways in which research in microbial iron metabolism have already found applicability in medicine. Microbial iron transport agents—the siderophores—are routinely used to remove excess metal in iron-storage diseases. Siderophores are also used as antidotes in cases of accidental iron poisoning in children. More recent work suggests that some accepted ideas about iron nutrition might have to be discarded. The prevalent attitude fostered by high-pressure advertising that "the more iron the better" may have to be reevaluated by a society that has become used to eating breakfast cereal with a magnet rather than a spoon. The common practice of iron supplementation in infancy must certainly be questioned, and the relationship of iron to fever and infectious disease should be a matter of intensive future research. In 1956, when Professor Neilands suggested to me that
working on microbial iron transport compounds might be an interesting problem for a Ph.D. thesis, neither of us could have guessed that this area of research might have so many future practical applications in the medical sciences.
References


