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Iron and Infection

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In the contest between the establishment of a bacterial or mycotic disease and the successful suppression of the disease by animal hosts, iron is the cation whose concentration... at present appears to be most important. (199)

Vulnerability to infection based on the individual's state of iron nutrition must be the net result of the effect of iron or the lack of it on microbial growth on the one hand and the host immune response on the other. (49)

The widespread notion that better than average nutrition leads to better than average resistance to infection appears to be a misconception. (91)

INTRODUCTION

Studies of (i) ability of normo-, hyper-, and hypoferremic hosts to withhold iron from invading microorganisms, (ii) capacity of virulent and avirulent microorganisms to acquire iron from hosts, and (iii) effect of the metal on phagocytosis and immune responses were reported sporadically in the four decades before 1970. Several reviews (33, 106, 201, 202) appeared early in the present decade, summarized the published data, and suggested work that remained to be done. Within the past 5 years, the quantity of papers in this area has exceeded that of the entire previous 45 years. The present review emphasizes the recent work and attempts to reconcile studies that are concerned with the host's vulnerability to infection because of iron excess on the one hand with those of iron deficiency on the other.

IRON-WITHHOLDING ACTIVITY BY HOSTS

Possible Mechanisms

Inasmuch as the growth requirement of approximately 0.4 to 4.0 μM iron is similar for cells of animals, plants, and microorganisms (202), it would appear at first glance to be unlikely that an antimicrobial defense mechanism consisting of iron withholding would be practical. Nonetheless, mammals, birds, reptiles, and perhaps all vertebrates make considerable metabolic adjustments during infection that have the effect of depriving invading microorganisms of iron. Six ways in which this feat might be accomplished are listed in Table 1.

With the understandable exception of infectious diseases in which either iron storage tissues or erythrocytes are damaged (11), the first proposed mechanism in Table 1 appears not to be used. An obvious reason for unsuitability of increased excretion of endogenous iron during microbial invasion is that stores of the metal certainly would need repletion after the illness had waned. Such repletion might be difficult, especially if the infectious episode had been protracted. Additionally, the excreted metal might enhance microbial growth in the skin or urinary tract. Increased iron content of skin, for example, has been proposed to be a factor in enhancement of susceptibility to fungal (104) and bacterial (137) infection. Still another reason for the probable lack of increased excretion of endogenous iron is that the process presumably would require an organic carrier. Thus, excretion would deplete the host not only of iron but also of iron-binding molecules and, as well, necessitate a selective mechanism for excretion of specific iron-rich compounds.

In contrast to increased excretion of iron during infection, a moderate decrease in intestinal absorption of the metal might be simpler to

TABLE 1. *Possible mechanisms whereby infected hosts could deprive microbial invaders of growth-essential iron*

Mechanism	Evidence that mechanism is utilized
(1) Increased excretion of endogenous iron in either urine, sweat, or bile and feces	No
(2) Decreased intestinal absorption of exogenous iron	Yes
(3) Contraction of iron in plasma compartment plus expansion of iron in storage compartment	Yes
(4) Prior stationing of host iron-binding proteins at potential sites of invasion	Yes
(5) Increased synthesis of host iron-binding proteins	Yes
(6) Suppression of synthesis of microbial siderophores	Presumptive

accomplish and have less impact on iron storage levels. Observations concerning the use by infected hosts of such a mechanism (no. 2 in Table 1) have been reported for humans, rats, and chicks. Decreased intestinal absorption of iron chloride, sulfate, and ascorbate has been observed in humans who were febrile because of natural infections (14, 43, 60) or diphtheria-pertussis-tetanus immunization (14). In a set of 19 children, for example, the mean level of absorption of an oral dose of ferrous ascorbate was 41.2% in the afebrile state, whereas in the same hosts in the febrile state it was only 15.1% (14). Similarly, endotoxin (55) and turpentine (88, 174) suppressed intestinal absorption of iron in rats; injection of these materials superficially resembles microbial invasion and thus triggers the overall inflammatory response.

It could be argued that infection and inflammation cause a nonspecific depression of intestinal absorption of all nutrients. That this is not the case is illustrated by considering zinc. Intestinal absorption of this metal was significantly increased in rats injected with either *Francisella tularensis*, endotoxin, or leukocytic endogenous mediator (LEM) (155, 156). Moreover, in chicks infected with *Salmonella gallinarum*, absorption of iron was decreased within 1 day, whereas total food uptake did not decrease until day 4 (90).

Evidence that infected vertebrates shift iron from plasma to storage (no. 3, Table 1) began to accumulate with the report (118) in 1932 that tubercular patients are hypoferremic. Since then, reduction of quantity of plasma iron has been observed in humans, other mammals, birds, and reptiles that have any of a great variety of infectious diseases as well as in mammals and birds in whom the inflammatory response has been induced by rheumatoid arthritis, cardiovascular accident, neoplasms, or injection of endotoxin or turpentine (10, 38, 44, 79, 80, 90, 154). Of course, if the condition causes a release of iron from stores (as in hepatitis) or from erythrocytes (as in hemolytic episodes in bartonellosis, malaria, leukemias, lymphomas, and Hodgkin's disease), plasma iron will rise rather than fall. In the absence of damage to storage tissues or erythrocytes, plasma iron level begins to decline early in the incubation period of the infectious disease (154); the extent of reduction in either acute or chronic illness typically is as much as 50% and often can exceed this in hosts who are extremely ill. Upon recovery, the level of plasma iron promptly returns to normal.

Plasma iron turns over about 10 times daily (209), and hypoferremia is achieved by an unknown mechanism(s) that suppresses return of the metal from the reticuloendothelial (RE) system (44) as well as accelerates flux of plasma iron into hepatic storage sites (10). Since in healthy hosts the quantity of plasma iron is only 0.4% of the amount of storage iron (209), withholding of $\geq$50% of the plasma metal by storage cells during infection apparently offers no logistical problem. However, since erythropoiesis is inhibited in infection, the storage tissues must also accommodate the iron that would otherwise have been utilized in hemoglobin production (10, 11). Thus, de novo ferritin synthesis might be required; fortunately, amino acids derived from catabolized muscle protein enter the liver during infection and presumably would be available as substrates for construction of the storage protein.

Parenteral administration of exogenous iron to infected persons does not restore the normal level in plasma; instead, the metal accumulates in RE cells of the liver and spleen (10, 79, 86). Iron-sufficient persons who have an infection- or inflammation-induced hypoferremia can be distinguished from iron-deficient persons by demonstration in the former of elevated levels of plasma ferritin that accompany the rise in RE storage of iron (93).

The shift of iron from the plasma to the storage compartment can be demonstrated also in uninfected normal rats who are injected with LEM (135, 195). This low-molecular-weight protein is released from activated leukocytes of infected hosts into their plasma within 2 h of inoculation with either microorganisms or endotoxin. LEM mediates acquisition by hepatic cells of iron, zinc, and a large number of free amino acids from plasma. Additionally, hepatic synthesis of ceruloplasmin, fibrinogen, and other acute-phase reactive proteins is stimulated (10).

Predictably, rats made leukopenic (e.g., with nitrogen mustard) and then injected with endotoxin showed impaired ability to become hypo-

ferremic, presumably due to decreased LEM release (100). In humans, systemic bacterial or parasitic infections produced high levels of mediator, whereas a lower amount was formed in patients with relatively localized bacterial infections (195). In contrast, viral infections stimulated only small increases in plasma concentration of the mediator. In volunteers who developed typhoid fever, quantities of circulating mediator became significantly increased before the onset of fever. An elevated plasma level of LEM is among the earliest indicators of the presence of systemic infection (195).

Non-heme iron-binding glycoproteins of the transferrin class are located at tissue sites of mammals and birds that are frequently or constantly threatened with microbial invasion (no. 4, Table 1). The first member (conalbumin; ovotransferrin) of the class was described in egg white in 1889 (16), but its iron-reversible ability to prevent microbial growth was not recognized until 1944 (173). Physiological quantities of other transition elements, complexed much less strongly than iron (16), are unable to reverse the microbiostatic action of these proteins (104, 108, 150). Conalbumin comprises 13% of egg white solids and, because of its ability to chelate iron, is considered to be the factor "most probably responsible for confining the infection of rot-producing bacteria to the shell membrane" (22).

Proteins of the transferrin class have now been found in the plasma of the majority of vertebrates studied as well as in secretions such as milk, nasal and bronchial mucus, tears, saliva, gastrointestinal fluid, hepatic bile, synovial fluid, urine, cervical mucus, and seminal fluid. The class member found in plasma mainly is transferrin (siderophilin); in secretions, transferrin and/or lactoferrin generally are present, depending on the host species (16). Additionally, lactoferrin is contained in the secondary granules of leukocytes; it is released at the site of microbial invasion and, after combining with iron, is bound and ingested by macrophages, provided that the latter have not been blockaded by particulate materials (189).

Each member of the transferrin class has a molecular weight of between 75,000 and 80,000 and reversibly binds two ferric and two bicarbonate ions per molecule (16). Each iron atom is linked to the protein apparently by three tyrosyl and two histidyl residues plus one bicarbonate ligand. Lactoferrin, at least at pH 6.4 to 6.7, has a greater avidity for iron than does transferrin (2) and possibly facilitates the transfer of iron from plasma to milk (16). Intravenous injection of lactoferrin into normal hosts induces very quickly a shift of iron from the plasma compartment to the RE cells. In an analogous manner, the protein derived from neutrophils in infected hosts apparently can function as a component of proposal no. 3 of Table 1 (189).

Under normal conditions, the transferrin class of proteins is relatively unsaturated with iron. Human plasma transferrin, for example, is approximately 25 to 35% saturated. Assuming a normal quantity of about 18 μM iron, a saturation value of 25%, and an association constant of $\geq 10^{30}$, it can be calculated that the amount of free ionic iron in human plasma is at least 10^{8}-fold less than that required for microbial growth. Similarly, lactoferrin of early human milk (5 to 10 days postparturition) is less than 5% saturated (172); moreover, in contrast to transferrin, it binds and holds adventitious iron tightly at pH values as low as 4.0 (189). Thus it is more capable than transferrin of withholding iron from potential microbial pathogens in the infant stomach, whose contents range from pH 5.0 to 6.5 (172).

The concentration of lactoferrin in early human milk is about 58 μM, and the iron-binding substance comprises 28% of the protein fraction; at 11 to 60 days postparturition, it falls to 20 to 27 μM and is 15 to 20% of the milk protein (15, 145). Nevertheless, lactoferrin of mature human milk is only 9% saturated with iron (164a) and does possess a considerable amount of iron-binding capacity (34). In contrast to bovine milk, whose iron-reversible action against human gut flora generally is limited to the period of colostrum production, that of human milk persists in samples obtained at various times after parturition (110). However, mature human milk possesses 2 to 3 mM citrate (and bovine milk 6 to 8 mM) (153), which, at pH 7.4, can make lactoferrin-bound iron available to bacteria unless blocked by bicarbonate (15, 81, 166).

An important function of plasma transferrin is the transport of iron to and among cells of the erythropoietic bone marrow, the RE system, the spleen, the liver, the small intestine, and muscle (209). Considerable in vitro evidence also has accumulated concerning its proposed microbiostatic function (106, 108). With lactoferrin, on the other hand, the microbiostatic role has been widely studied (15, 18, 34, 81, 166), but there is little information concerning its possible function in facilitation of iron absorption in nurslings (69). A nutritional role of human lactoferrin is suggested by the observation (6a) that infants absorb 49% of iron from human milk but only 4% from bovine milk. However, evidence that human lactoferrin fails to promote iron absorption has been reported (127a). Conalbumin apparently serves only a microbiostatic role and has no iron-ferrying function. The iron necessary for growth of the avian embryo has been depos-

ited by the hen in the yolk. The considerable quantity of yolk iron (e.g., 1 mg per chicken egg [188]) is not accessible to microorganisms that attempt to advance through the shell membrane unless, of course, the vitelline membrane has been ruptured by addling.

Inasmuch as preformed iron-binding proteins serve a useful microbiostatic role at potential sites of invasion, we might predict that increased synthesis of such compounds would occur in episodes of microbial attack (proposal no. 5 of Table 1). During infections in normal humans, however, as the hepatocyte iron-ferritin pool increases, hepatic anabolism of transferrin becomes slightly lower than catabolism (96) so that a decrease of as much as 15 to 20% of normal levels can ensue (195). In contrast, in mice infected with *Listeria monocytogenes* (185) or with *Mycoplasma arthritidis* (62), the concentration of plasma transferrin increased markedly. In the latter case, plasma iron decreased by 33%, whereas total iron-binding capacity was elevated by 66%. That transferrin, rather than lactoferrin or ferritin, was the substance that was increased in the plasma of infected mice was established by immunoelectrophoretic methods (185). The percentage of iron saturation of plasma transferrin in mice normally is twice that of humans (106); thus the mice may be required to develop both hypoferremia and hypertransferrinemia in order to abort infections promptly (62). Moreover, the hypertransferrinemia might also compensate for the inability of infected mice to develop hyperthermia. The possible host-protective action of the latter is discussed below.

An interesting example of compensation in humans also has been described. Four agammaglobulinemic patients had hypertransferrinemia sufficient to result in double the unbound iron-binding capacity of the controls (129). As compared with control sera, the test samples also had twice as much bacteriostatic potency; moreover, the latter was independent of complement and was reversed by exogenous iron.

The accumulation of lactoferrin at sites of infection in human and other hosts is well established but often represents secretion of preformed molecules by neutrophils that have migrated to the site rather than an increased rate of synthesis. For example, in 39 arthritic patients, the mean concentration of lactoferrin in 12 noninflammatory synovial fluid specimens was 14 nM, whereas in 27 inflamed samples it was 330 nM (13). From a calculation of the number, lactoferrin content, and rate of disruption and turnover of neutrophils in inflamed specimens, the authors concluded that the accumulated iron-binding protein was derived from the immigrant defense cells rather than synthesized de novo by synovial membrane cells.

Likewise, in nine patients with bacterial meningitis or pneumonia, plasma lactoferrin increased within 2 days of onset from a mean of 4 nM to 15 nM; as in the study of arthritic patients, the increase was reported to be mainly derived from disrupted neutrophils (83). Indeed, within 60 min after onset of phagocytosis by neutrophils, up to 40% of lactoferrin has been observed to be degranulated and approximately 86% of that has been observed to have been released from the cells (115).

Nevertheless, one tissue exposed to microbial invasion in which the rate of synthesis of lactoferrin apparently does increase has been identified: the bovine mammary gland. Within 90 h of the onset of either experimental or natural mastitis induced by *Escherichia coli*, the concentration of secreted lactoferrin was increased 30-fold above normal (84). Based on the number of neutrophils that had accumulated in the infected area, the authors calculated that not more than one-tenth of the extra lactoferrin could have been derived from the defense cells. The remainder was considered to be the result of the specific response of secretory tissue to an infectious process (84).

In healthy cows, the quantity of nonneutrophilic, secretory lactoferrin begins to increase within 2 days after cessation of regular milking and to plateau in 3 to 4 weeks. In one study (181), the mean amount of lactoferrin in milk of 15 cows was 3.1 μM, whereas in the lacteal secretion in the 4th week of involution it was 308 μM. In fact, in one animal, the concentration attained was 1,480 μM. The authors suggested that the high amount of lactoferrin and low quantity of citrate in involuted mammary gland tissue contributes to the considerable resistance to bacterial invasion of that site (18, 84, 181).

The final proposed mechanism (no. 6, Table 1) whereby hosts might withhold iron is that of interference with a specific component of the microbial iron acquisition system. The latter will be discussed more fully below; at this point, we will consider briefly presumptive evidence concerning the ability of hosts to suppress formation of a key component of the system, namely, the microbial iron transport cofactors. In vitro studies showed parallel growth rates at 37 and 41°C for strains of *Salmonella typhimurium* (75), *E. coli* (108), and *Aeromonas hydrophila* (80) and at 20 and 28°C for *Pseudomonas* sp. (74); efficient synthesis of siderophores occurred in each case only at the lower temperature. At the higher temperature, additional iron or preformed siderophores were required to obtain growth.

In in vivo studies in which lizards (105) and fish (56) were inoculated with *A. hydrophila*, hosts that were permitted to raise their body temperatures (by means of exogenous heat sources) had much better survival than did control hosts that were prevented from developing a hyperthermia. Infected lizards with raised body temperature achieved a greater hypoferremia than did infected controls; injection of sufficient exogenous iron to create a hyperferremia markedly lowered the number of survivors (80). Evidence for proposal no. 6 is categorized as presumptive rather than definitive because the actual quantity of microbial siderophores produced in normo- and hyperthermic host tissues has not yet been measured. Nevertheless, one of the beneficial aspects of fever does appear to be that of exacerbation of the iron famine that thwarts potential microbial invaders.

Fever temperatures also suppress microbial formation of such secondary metabolites as exotoxins and other factors of virulence (203). With bacteria, this effect of fever may be simply a result of interference with the supply of iron, since synthesis of bacterial secondary metabolites has a much narrower tolerance for range of concentration of the metal than does growth (19, 200). Fortunately, hyperthermia appears to be compatible with the host mechanisms listed in no. 2, 3, and 4 of Table 1; for example, rabbit pulmonary macrophages accumulate iron equally well at 40 and 37°C (121).

Suppression by Hyperferremia

If vertebrate hosts do indeed restrain microbial growth by withholding iron, this form of "nutritional immunity" should be overcome readily and specifically by stressing the host with excess iron. A large number of in vitro and in vivo studies have been reported that have demonstrated that added iron enhances the ability of designated strains of microorganisms to grow in host cells, tissues, and/or fluids. The genera most often used are listed in Table 2, and examples of data from representative in vivo studies are contained in Table 3. Of course, not all strains of a given microbial species respond equally well to added iron; reasons for strain variation are explored below.

In general, a quantity of iron that is sufficient to double the saturation value of the iron-binding protein in the particular system will have marked effect on the ability of various microbial strains to multiply in that environment. In some cases, the minimal size of the inoculum that would be required for growth in the control system can be lowered by 4 or 5 log units. Moreover, a variety of sources of iron are effective: ferric and ferrous salts, iron sorbitol, ferric ammonium citrate, iron dextran, heme, hematin, hemin, hemoglobin, and ferritin. Even the virulence-enhancing action of mucin has been demonstrated to reside in its content of iron (40, 72).

TABLE 2. *Examples of genera that contain strains whose growth in body fluids, cells, tissues, and/or intact vertebrate hosts is stimulated by excess iron*

Fungi and protozoa	Gram-negative bacteria
Candida (42, 63)[a]	*Aeromonas* (80)
Plasmodium (144)	*Alcaligenes* (21)
Trichophyton (104)	*Enterobacter* (21)
Gram-positive and acid-fast bacteria	*Escherichia* (33, 34, 66)
	Klebsiella (137)
Bacillus (77)	*Neisseria* (40, 151)
Clostridium (32, 33)	*Proteus* (21)
Corynebacterium (54)	*Pseudomonas* (21, 35, 68)
Listeria (186)	*Salmonella* (47)
Mycobacterium (107, 206)	*Shigella* (47)
Staphylococcus (77, 171)	*Vibrio* (98a)
	Yersinia (37, 192)

[a] Numbers in parentheses refer to references.

Since experimental methods of saturating host iron-binding proteins with iron are so effective in enhancement of microbial growth, we can predict that natural methods likewise should increase the incidence and severity of infectious diseases. Observations concerning this prediction have been extensively documented; examples are contained in Table 4. Of course, persons with the underlying conditions contained in the table who are not simultaneously exposed to potentially virulent microbial strains will be able to avoid infection. On the other hand, many patients suffering from the conditions listed in Table 4 will not only be deficient in nutritional immunity but may also be compromised in some aspect of humoral or cell-mediated immunity. Such persons would be expected to have even less resistance to infection than those deficient only in nutritional immunity. Moreover, in some conditions, more than one component of nutritional immunity is damaged. In sickling, for example, elevated plasma iron derived from a 30-fold increase in plasma hemoglobin (147) cannot readily be lowered by the RE system because the latter has been blockaded by erythrocyte membranes. Nevertheless, even when healthy humans with no known abnormalities are stressed with excess iron (8, 9, 65), their resistance to microbial invasion becomes seriously impaired.

Even persons who ingest insufficient amounts of iron can suffer from a defect in nutritional immunity if their ability to synthesize iron-binding proteins is injured. In kwashiorkor, for example, humoral immunity is adequate in most patients (124, 125), but plasma transferrin may be as low as 3.5 μM and thus be $\geq$100% saturated (124). Administration of iron without prior feeding of protein to rebuild transferrin levels in such

TABLE 3. *Examples of effect of iron on normal hosts exposed to microorganisms*

Disease	Host	Iron administration			Result			Reference
		Amt (mg/kg)	Route[a]	Time[b]	Time[b]	Controls	Iron-stressed	
						(LD_{50} × 10,000)		
Gonococcal infection	Chicken embryos	1.25	i.v.	t_0	t_1	3.47	0.01	(151)
Meningococcal infection	Mice	16.0	i.p.	t_{-1}	t_3	1,000	0.23	(40)
						(CFU[c]/g of kidney × 10,000)		
Coliform pyelonephritis	Rats	4.0	i.m.	t_0	t_5	0.1	100	(66)
Corynebacterial pyelonephritis	Rats	10.0 (×6 = 60)	i.m.	t_1–t_6	t_7	4.8	12,000	(54)
						(CFU/g of muscle × 10,000)		
Gas gangrene	Guinea pigs	5.0	i.m.	t_0	$t_{0.66}$	0.2	1,000	(32)
						(CFU/g of liver × 10,000)		
Listeriosis	Mice	4.0 (×3 = 12)	i.p.	t_{-1}, t_0, and t_1	t_3	0.2	140	(186)
						(% parasitemia)		
Malaria	Rats	160.0	i.m.	t_0	t_{12}	20	57	(144)
						% iron-saturated transferrin		
						43	99	(144)
						(% deaths)		
Systemic candidiasis	Mice	10.0	i.p.	t_0	t_1	10	95	(63)
Tuberculosis	"Immune" mice	5.0 (×18 = 90)	i.p.	t_0–t_{17}	t_{18}	5	60	(107)
Staphylococcal infections	Mice	17.0	i.p.	t_0	t_3	0	92	(77)
Coliform infections	Mice	4.0	i.p.	t_0	$t_{0.75}$	0	92	(77)
						(cases per thousand)		
Gram-negative bacterial septicemias and meningitis	Human neonates	10.0 (×5 = 50)	i.m.	—[d]	—[e]	2.7	17	(8)
Coliform meningitis	Human infants	10.0	i.m.	—[f]	—[e]	0.3	2.1	(65)
						(mean survival [days])		
Salmonellosis	Mice	—[g]	p.o.	NA[h]	NA	7.4	3.4	(179)

[a] i.v., Intravenous; i.p., intraperitoneal, i.m., intramuscular; p.o., oral.
[b] t_0 = day on which microorganisms were inoculated into hosts.
[c] CFU, Colony-forming units.
[d] Iron injected daily between 2nd and 7th days of life; bacteria were derived from normal contaminating flora.
[e] Clinical disease developed within 1 week of iron administration.
[f] Iron injected in single dose sometime between 7th and 47th days of life; bacteria were derived from normal contaminating flora.
[g] 50 mg of iron (as ethylenediametetraacetic acid chelate) per kg of food before and during illness. Neither $FeSO_4$ nor iron-nitrilotriacetic acid chelate was active in this system.
[h] NA, Not applicable.

patients has exacerbated their susceptibility to bacterial infections (124). Plasma from untreated victims of kwashiorkor supported growth of *Staphylococcus aureus*; the plasma could be rendered bacteriostatic by addition of apotransferrin (123).

In a similar study, hyperferremic plasma from patients in hemolytic episodes of leukemia supported excellent growth of *Candida albicans*, whereas normal human plasma was mycostatic. The former could be converted to a "static" environment simply by adding apotransferrin;

the latter could be converted to a "growth" environment merely by adding iron (42). In an analogous experiment, hyperferremic blood from sickling patients supported a 31-fold increase in growth of *S. aureus* over samples from normal persons (132).

In a different study (9), venous blood was obtained from seven healthy human 1- to 54-day-old neonates immediately before and 24 h after intramuscular injection of 20 mg of iron (as iron dextrans) per kg. No differences in the two samples were detected in either ability of leukocytes to reduce tetrazolium salts or to kill *E. coli* or in the opsonizing property of the sera. In contrast, leukocyte chemotaxis and the bacteriostatic effect of the post-treatment sera (which were more hyperferremic than the pretreatment samples) were markedly reduced against *E. coli* (9). As indicated in Table 3, iron-stressed neonates have lowered resistance to bacterial infections (8, 65). Not surprisingly, these various in vivo and in vitro observations made with humans and human products are quite similar to those obtained earlier with lower animal systems (106).

In some cases, however, iron stress is not even contemplated by the investigators as a possible determinant in the initiation of infectious disease. For example, in one study (23), eight premature infants were fed a bovine milk formula that contained 25 μM iron; the amount of lactoferrin was possibly sufficient, if 100% saturated, to bind 6 to 30 μM. Eight other premature infants, very similar to those in the first set, were fed an elemental formula that contained, as assayed by the food processor, 214 μM iron. The source of nitrogen in the latter formula was casein hydrolysate; no lactoferrin was present. Necrotizing enterocolitis developed in only two of the eight infants in the first group but in seven of the eight of the second set. The authors attributed the infection-predisposing action of the elemental formula to its somewhat higher osmolality as compared with the cow milk formula.

The ability of severe viral infections to render patients temporarily highly susceptible to bacterial invaders has long been known. Since fulminating viral illness can induce hemorrhage (45, 187), one of the components of enhanced susceptibility to bacterial growth may simply be a surfeit of iron derived from extravasated hemoglobin.

Enhancement by Hypoferremia

If vertebrate hosts do, in fact, attempt to withhold iron from microbial invaders, this form of nutritional immunity should be enhanced by administration of various hypoferremic agents just before or at the time of invasion. Agents that might be effective include sham-infectious material (e.g., endotoxin or other microbial cell surface products), attenuated microorganisms (e.g., BCG), hormone-like compounds (e.g., LEM), host iron-binding proteins, and possibly microbial siderophores, provided that the latter could not be utilized for iron acquisition by the pathogen under consideration.

In in vitro studies, guinea pigs were rendered hypoferremic by injection of either *E. coli* endotoxin, cell wall extracts of *Mycobacterium tuberculosis*, or living attenuated tubercle bacilli (BCG); their sera gained bacteriostatic activity in proportion to loss of iron. Moreover, the activity was neutralized simply by adding sufficient metal to restore the iron concentration to normal (106). These observations were confirmed in a study of growth of *C. albicans* in sera of mice that had been injected with *E. coli* endotoxin (63).

In experiments in vivo, endotoxin injected into mice before inoculation with *S. typhimurium* (47) or with *C. albicans* (63) enhanced host survival; the action in each case was neutralized specifically by injection of sufficient iron to restore normal plasma levels. Likewise, the survival of rats inoculated with *S. typhimurium* was considerably prolonged in those animals rendered hypoferremic by prior injection of either endotoxin or LEM (100). The 50% lethal dose (LD_{50}) values of virulent strains of *Neisseria gonorrhoeae* for chicken embryos were raised approximately 300-fold by inclusion of 62 μM apoconalbumin with the bacterial inoculum; predictably, the host-protective action of the protein was prevented by saturating it with iron (151). Experiments in which infected hosts were exposed to exogenous microbial siderophores will be described later.

A discussion of the possible host-protective effect of hypoferremia would be incomplete in the absence of consideration of quite direct but medically unsound methods for obtaining a low iron status: inadequate levels of iron in the diet or excessive loss of blood. The results of five studies in rodents, one in chicks, and approximately fifteen in humans are available. In the initial animal study (103), the rate of death and cumulative mortality of mice infected intraperitoneally (i.p.) with *S. typhimurium* were unaffected by withdrawal of sufficient blood to obtain a marked anemia.

In a nutritional, rather than hemorrhagic, study, rats were given various amounts of dietary iron and then fed *S. typhimurium*; 100% of the hosts that received no detectable iron survived (6). Paradoxically, the least resistant (≤50% survival) were the marginally iron-defi-

cient set, i.e., those that received 5 to 25 mg of iron per kg. Seventy-five percent of iron-sufficient hosts (fed 35 mg/kg) survived. The authors commented that "this observation is at variance with the intuitive bases of many nutrition programs that any improvement in nutritional status is laudable." The paradox may have resulted from the introduction of the bacteria into the intestine rather than directly into the aseptic peritoneal cavity. The different amounts of iron in the gut might have stimulated or suppressed synthesis of siderophores by various indigenous flora; in turn, the metal-binding compounds might have been able to control growth of the pathogen. Studies in germfree rats would assist in exploration of this possibility. As we will learn in the next section, siderophores may stimulate or suppress growth of invaders depending on the capacity of the bacteria to utilize the iron transport cofactors.

In a subsequent study, three sets of mice were injected i.p. with *S. typhimurium* (162). Set I was fed a normal diet so that at the time of infection, mean plasma transferrin iron saturation was 80% and hemoglobin (Hb) value was 12.8 g/dl. Set II was fed a diet that contained <5 mg of iron per kg but was given a single i.p. injection of 1 to 1.5 mg of iron per mouse at 10 to 14 days before infection; at the latter time, mean plasma transferrin iron saturation was 55% and Hb value was 11.7 g/dl. Set III was fed <5 mg of iron per kg so that at the time of infection, mean plasma transferrin iron saturation was only 19% and Hb value was 10 g/dl. Thus a mild iron deficiency status was achieved in set III. Predictably, survival in the three sets should be inversely proportional to transferrin saturation; the actual survival values were: set I, 10.8%; set II, 19.7%; and set III, 58.5%.

In a third animal study of dietary iron deficiency and infection (53), rats were rendered highly rather than marginally iron deficient; the mean Hb value was 13.8 g/dl for controls and only 5.4 g/dl for iron-deficient hosts. The latter would be expected to have impaired resistance (see discussion below), and the results confirmed this expectation. The LD_{50} value of a strain of *Streptococcus pneumoniae* injected i.p. was 3 log units less in the iron-deficient than in the control hosts. In a different study, nutritional iron deprivation in rats was severe enough to delay antibody synthesis; predictably, their resistance to *Trypanosoma lewisi* was lowered (114).

Chicks aged 15 days were fed a single dose of *S. gallinarum* (180). Hosts that were simultaneously injected intramuscularly with 20 to 200 mg of iron per kg of body weight had 2 log units fewer bacteria per g of liver and approximately twice the survivors as infected hosts deprived of iron. The latter had Hb values 6 to 16% less than the iron-supplemented birds, depending on the type of diet. As with the study on rats that were fed the bacterial inoculum (6), the experiments with chicks should be repeated in germfree birds to exclude possible stimulation by iron of avirulent intestinal flora that might be producing anti-*Salmonella* siderophores. Of course, in germfree hosts, the size of the bacterial inoculum would need to be lower than that used in conventional birds.

To what extent might a mild nutritional deficiency of iron protect humans against infection? The hypothesis that starvation suppresses and refeeding activates infections as an essential part of an ecological balance between hosts and pathogens has been proposed (141–143). Of 181 persons who shifted abruptly from a "famine" diet to a "hospital" diet, 41% developed clinical *Plasmodium falciparum* malaria in 5 days; their plasma iron and transferrin iron saturation had risen dramatically within 2 days of onset of refeeding (144). In another study, these authors observed recrudescence of brucellosis and tuberculosis, as well as malaria. For example, within 2 to 3 weeks after onset of refeeding, long-inactive tubercular lesions broke down and formed sinuses and abscesses (143). It was proposed that mobilization of iron from stores after resumption of an adequate diet temporarily exceeded the rate of host utilization of the metal.

In a different study, of persons with Hb values of <10 g/dl, 60% of 65 patients with adequate iron stores had bacterial infections, whereas only 8% of 87 patients with low iron stores were infected (131, 132). Unfortunately, plasma iron and transferrin iron saturation values were not obtained, nor were data reported concerning the incidence of infection in persons in the same environment but whose Hb values were ≥10 g/dl.

In other studies, markedly iron-deficient (Hb < 10 g/dl) persons were in some, but not all, cases more susceptible to infection than were normal individuals. For example, severely deficient persons who had no storage iron (89) or whose mean Hb value was 7.6 g/dl (67) had an increased incidence of mucocutaneous candidiasis that generally was reversible upon iron repletion. In contrast, only 1 of 12 persons with a mean Hb of 9.0 g/dl but 4 of 12 iron-sufficient controls had oral candidiasis, and iron repletion failed to aid the deficient patient (193). Moreover, no significant difference in iron status was observed between 74 women with and 26 women without genital candidiasis (58a). Young adults with very mild iron deficiency had the same incidence of upper respiratory infections as nor-

mals both before and after iron repletion (190).

In pregnancy, anemia is considered to be present if the Hb value is <11 g/dl (4). Bacteriuria was observed in one study (76) to be twice as common in pregnant women whose Hb values were <10.4 g/dl as in those whose Hb levels were higher. In two other surveys of large numbers of pregnant women with Hb values between 8 and 15 g/dl, the mean Hb levels of the bacteriuric patients were 0.3 to 0.4 g/dl less than in the normal groups (1, 170). However, in a fourth study of 5,000 pregnant women, 11.9% of the 4,735 normals but only 9.4% of the 265 bacteriurics had Hb values <11 g/dl (117).

Anemia is likewise considered to be present if a child aged 6 months to 6 years has an Hb value <11 g/dl (4). In approximately 500 3- to 24-month-old children, the incidence of infection was unrelated to Hb values in the range 9.25 to 14.28, with mean values at various monthly ages of 11.5 to 12.4 (36, 71). However, in more severely iron-deficient children (mean Hb values of 9.0 to 10.5), administration of iron to restore normal levels did increase resistance to infectious disease (127).

In a later study of 1,048 children between zero and 18 months, the control group had mean Hb values of 10.3 to 11.3, whereas the group fed supplemental iron had means of 11.5 to 12.3 (3). Respiratory infections were reported during the entire study at a mean of 17.5% of the control group and in only 12.3% of the iron-enriched set. Unfortunately, microbiological data were neither collected nor reported; rather, complete reliance was placed on recollections of the mothers of the children who were interviewed at intervals of 1 to 3 months. It is not known whether any of the infectious episodes in either group were severe enough to require medical attention.

In view of the important functions of iron in host physiology and of the contingency that severe iron deficiency might enhance susceptibility to microbial attack, it is obviously too dangerous to attempt to increase host resistance to infection by creation of a mild iron-deficient nutritional status. Whether or not it may become medically acceptable to intensify the natural transient hypoferremia that develops during the incubation and acute phase of an infection (e.g., by administration of bacterial cell wall products, LEM, lactoferrin, or siderophores) remains unanswered.

INHIBITION OF MICROBIAL IRON ACQUISITION

Possible Sites

Cells have evolved specific high-affinity systems to acquire each growth-essential mineral (177, 204); those involving iron acquisition are becoming fairly well characterized. Microbial iron transport cofactors (siderophores, siderochromes) belong to at least two chemical families: secondary hydroxamic acids (e.g., ferrichrome, ferrioxamine B) and phenolic acids (e.g., enterochelin) (39). Since the affinity constant for iron of microbial siderophores is comparable to that of the transferrin class, the microbial chelators are capable of extracting the metal from the proteins and making it available to microbial invaders. Of course, the quantity extracted is influenced by such factors as the extent of iron saturation of the proteins, the pH of the environment, the presence of citrate and/or bicarbonate (160), and the molar ratio of siderophore to host protein (106). For example, a mycobactin/transferrin molar ratio of 1:50,000 was needed to obtain sufficient iron for bacterial growth if the protein was 100% saturated; in contrast, at 30% saturation, a ratio of 1:100 was required (106).

After synthesis, the desferrated cofactors are released by microbial cells into the environment and subsequently reassimilated as ferric chelates. Special receptors and transport components at or within the cell envelope are required for uptake of the chelates. The latter then serve as an intracellular source of iron; the metal is withdrawn from the siderophores as needed by reducing it to the ferrous state and, in the case of the phenolates, by enzymatic hydrolysis of the ligands. Furthermore, microorganisms often can utilize hydroxamates or phenolates produced by cells of other microbial genera (39, 136). Additionally, some strains of *E. coli* can use citrate to facilitate iron uptake (39, 81). Thus various siderophores may be bound and transported by independent systems within a single organism. Presumably, a microbial newcomer that enters an environment in which other organisms have been multiplying and excreting siderophores would be unable to initiate growth unless it first could bind, transport, and withdraw iron from the extrinsic chelates (39).

In the case of enterochelin synthesis and ferric-enterochelin uptake in *E. coli*, at least 13 genes are involved (177). "The investment of cellular protein and energy into enterochelin synthesis demonstrates aptly the important role of iron limitation in governing bacterial growth and the lengths to which bacterial cells will go to obtain what little iron is available in the environment" (177). In view of the number of sequential processes necessary to effect iron extraction and uptake, it should be possible to disable the system by interference at one or more sites that cannot easily be bypassed. In

this section, we will review knowledge concerning a few of the sites and suggest possible applications in control of infections.

Let us first consider the spectrum of response of microbial strains to the addition of iron and/or desferri-siderophores to host fluids, cells, or tissues. On the one hand, highly virulent strains have LD_{50} values so low that the latter cannot further be reduced by addition of the exogenous nutritional stimulants. Nevertheless, these strains apparently obtain iron for growth from the host environment since addition of exogenous host iron-binding proteins in some cases raises their LD_{50} values (151) and incubation in the presence of an excess of such proteins causes them to die (109, 110). Competency to acquire iron has been correlated with ability to cause disseminated rather than localized infections (158a).

On the other extreme are strains that have very high LD_{50} values that cannot be lowered by exogenous iron (30, 137); apparently, such strains lack biochemical attributes not related to iron nutrition that preclude normal rates of in vivo growth. The majority of strains tested thus far have either moderate or high LD_{50} values that can be lowered, to some extent, by an exogenous supply of either iron or utilizable siderophores (30, 92, 98, 107, 109, 137, 167, 192). In some studies, enhancement of virulence was affected by the route of microbial inoculation (30, 130) or by the kind of host and number of passages (35, 68); in all cases, the time at which iron or siderophore was supplied was important. If the nutritional stimulants were delayed, the microbial inoculum in some cases began to die because of lack of available iron (109, 110). Alternatively, if the inoculum could produce its own siderophores promptly, delayed addition of stimulants had a lesser effect on severity of the ensuing infection than when provided at the time of inoculation (33, 108, 137). In the following discussion, we will be concerned mainly with methods for preventing highly and moderately virulent strains from acquiring iron from normal hosts.

Suppression of synthesis of siderophores. Mutants have been described that are blocked at various steps in siderophore synthesis (39). Additionally, synthesis by wild strains can be suppressed by environmental factors. As indicated earlier, a number of bacterial strains have been observed to grow equally well at each of two temperatures, but to produce little or no siderophores at the higher temperature (74, 75, 80, 108). A second environmental factor that may affect siderophore synthesis of some microbial strains is oxygen. Microaerophiles such as *Spirillum volutans* and *Campylobacter fetus* grew well in 17% oxygen if provided a dihydroxyphenolate and in 21% oxygen if given both the phenolate and additional iron, whereas a supply of neither was required for growth at 6% oxygen (25). Conversely, in the complete absence of oxygen, unknown siderophores that have a selective affinity for ferrous rather than ferric iron might be needed (113). Enterochelin was neither produced (168) nor accumulated from the environment (161) by *E. coli* grown under anaerobic conditions.

A third factor observed to depress siderophore synthesis (or release) is immunoglobulin G (IgG) specific for the whole bacterial organism (167). In contrast to suppression of their synthesis, ability of the siderophores to withdraw iron from transferrin is not affected by either elevated temperature, oxygen excess, or antibacterial IgG.

Iron itself has long been known to depress excess siderophore production (113); moreover, it inhibits synthesis of an outer membrane polypeptide(s) that binds the ferric chelates (61). Nevertheless, in high-iron environments, the quantity of the nonrepressible residue of siderophores is greater than the concentrations required for growth of anautosequesteric mutants (113). In some microbial systems, the quantity of siderophores produced was observed also to be altered by such trace metals as manganese, cobalt, and zinc (113, 164).

Suppression of binding of ferric chelates. Mutants that are unable to use their own or foreign siderophores may have defective binding sites in their walls or in their inner or outer cell membranes. Some of these latter sites in wild strains also are utilized by various bacteriophages and bacteriocins (28). The "latter two destructive entities have capitalized upon a site so essential for cell metabolism that it cannot be modified significantly without serious loss of the capacity to accumulate iron" (39). As with synthesis mutants, binding mutants are able to grow in in vitro environments that have unnaturally large amounts of ionic iron, but they could not be virulent in any but the most iron-overloaded or transferrin-deficient kinds of host.

That lipopolysaccharide (LPS) might be involved in binding ferric chelates in the outer membrane of gram-negative bacteria was suggested by the demonstration that LPS extracted from a virulent strain of *E. coli* could facilitate the use of transferrin iron by an avirulent strain. In contrast, LPS from the avirulent strain was inactive in this system (110). Furthermore, in a set of eight mutant strains of *S. typhimurium* that produced decreasing amounts of the polysaccharide core component of LPS, the ability to bind complexes of transferrin-iron-enterochelin was correspondingly diminished (108,

112). Indeed, mutants that completely lacked polysaccharide could make no use of the iron complexes. Likewise, such mutants were avirulent in both normal and iron-stressed mice. In serum supplied with iron citrate, each of the eight strains grew well.

Other mutants of *E. coli* have been described in which enterochelin was synthesized but not transported across the outer membrane. Apparently, the siderophore could sequester iron present between the outer and cytoplasmic membranes and be returned to the cell interior in the ferrated form (70).

In *Mycobacterium tuberculosis*, the quantity of lipid in the cell surface is important in the acquisition of ferrated mycobactin. A nonvirulent strain synthesized as much of the hydrophobic siderophore as did a virulent strain, but the lesser lipid content of the former prevented efficient binding of the chelate. Mycobactin could readily be extracted by nonionic surfactants from the avirulent but not from the virulent strain (108).

Possible Applications

The earliest attempt to interfere with the microbial acquisition of iron was begun in 1945 in the laboratories of Imperial Chemicals, Ltd. (182). Mycobactin, the first bacterial growth factor to be clearly identified, had been described in 1912. Although the mechanism of stimulation of mycobacterial growth by mycobactin was not known in 1945, it was proposed that the structure be elucidated and analogs synthesized. Since mycobactin was recognized to be unable to stimulate growth of animals and humans, the proposed analogs were predicted to be neither toxic to hosts nor neutralized by an excess of growth factor in host tissues. Unfortunately, the complex chemical nature of the mycobactin hydroxamates made it difficult to synthesize analogs that were sufficiently close in structure to the growth factor to be antagonistic; the compounds that were produced failed to inhibit *M. tuberculosis*. Moreover, during the post-1945 decade in which mycobactin was being characterized, a number of antitubercular compounds became available; thus medical and economic incentives for the analog approach became weakened.

Ironically, between 1947 and 1960, random screening of actinomycetes for novel antibiotics yielded such classes of competitive inhibitors of non-mycobactin hydroxamate siderophores as the albomycins and ferrimycins (149). These compounds, termed sideromycins, consist of a siderophore moiety plus a side chain. The structural resemblance of these antibiotics to siderophores probably permits them to use the same uptake system, and they are believed to compete for binding sites rather than for iron (149). Within the cell, the antibacterial action of the sideromycins apparently is caused by the pendant group. Unfortunately, resistance is readily acquired; moreover, the compounds are chemically unstable.

Since cells of some microbial strains cannot use specific natural siderophores to obtain iron, it should be possible to depress their virulence by administering such compounds to the infected host. Evidence confirming this prediction has been reported for both normal and iron-overloaded mice infected i.p. with *S. typhimurium* (98). A mutant strain was unable, in low iron culture, to synthesize enterobactin or to use desferrioxamine (DF) but was able to utilize exogenous 2,3-dihydroxybenzoic acid (DHB) to acquire the metal. In both types of host, virulence of the strain was unaltered by iron overload, lowered by DF, and raised by DHB. In contrast, a wild strain (that could synthesize siderophores in culture) was enhanced in virulence not only by iron overload, but also by injection of infected hosts with either DF or DHB.

Virulence of other bacterial strains likewise has been lowered by administration of siderophores to infected animals. For example, the LD_{50} value of a strain of *L. monocytogenes* for mice was enhanced 30-fold by injection of DF (186); apparently this strain was impaired in siderophore synthesis or binding because it required 1,800 μM iron for complete growth in culture. In a different set of experiments in mice, virulence of a strain of *S. typhimurium* injected i.p. was lowered by i.p. inoculation of a nonvirulent strain 1 day earlier plus inclusion of dietary enterochelin from 2 days before to 3 days after inoculation of the avirulent strain (196, 197). In culture, the latter produced only 20 to 33% of the quantity of siderophores formed by the virulent strain. Possibly, nutritional enterochelin sufficiently stimulated growth of the avirulent strain to trigger the set of hypoferremic responses described earlier so as to protect the host from the virulent cells. Caution is required in this type of procedure; in one experiment, enterochelin caused the avirulent strain to kill the hosts (196).

In an aquatic (nonhost) environment, the hydroxamate siderophore of *Anabaena flos-aquae* prevented growth of *Scenedesmus* (140). The inhibition was reversed by excess iron.

Might it be possible to develop in vitro tests that would predict which siderophores might be unusable by a specific pathogen isolated from a patient? Agar media indicator systems have been described for siderophore production and

utilization in which either wells (108), cups or beads (136), or paper strips (149) are used; these could readily be modified for such a purpose. A system consisting of (i) the pathogen obtained from the patient, (ii) a body fluid from the infected site, (iii) agar culture medium, and (iv) prospective siderophores could be observed for pathogen growth at the same time that antibiotic susceptibility tests are being performed. Of course, if an unusable siderophore for the specific pathogen were to be detected, its use in the patient would need to be compatible (and hopefully synergistic) with the chemotherapeutic agent that is being administered.

In addition to measurement in vitro of pathogen growth in the presence of prospective siderophores, it would also be useful to monitor exotoxin production. As indicated earlier, the synthesis of bacterial exotoxins has a considerably narrower tolerance for range of concentration of iron than does bacterial growth (19, 200). Conceivably, a poorly utilized siderophore might permit growth of the pathogen but could suppress toxigenesis in both an in vitro test and in vivo; such a compound might be a helpful adjunct to antibiotic therapy.

To what extent might active or passive immunization procedures be developed to suppress microbial acquisition of iron? Among possible kinds of antigens are siderophore haptens and receptor-binding complexes. Antibodies to the mycobactin hapten were not detected in animals immunized against *M. tuberculosis* but might have been present at a very low concentration (106). A partially purified outer membrane receptor protein(s), specific for ferrichrome but not for either DF or enterochelin, has been extracted from *E. coli* (120). Antibodies to such an extract might interfere with the acquisition of ferrated ferrichrome not only by *E. coli* but by other bacteria as well. The inhibitory action of IgG against whole bacterial cells on siderophore synthesis (167), rather than uptake, has previously been cited.

Mutant strains that are defective in acquisition of iron in vivo have been proposed as possible immunizing agents (152). In *Neisseria*, for example, desirable mutants would be those that could colonize mucosal surfaces to stimulate local immune response but would be unable to disseminate and cause systemic infection. Of course, such mutants would need also to be restricted in utilization of siderophores produced by indigenous flora. Quantitative and qualitative determinations of siderophores in the diet and in various tissues and fluids derived from contaminated areas of the body should be made in order to evaluate the possible danger of "microbial vitalization" (110), i.e., the ability of siderophores produced by one strain to become available to a second strain.

Finally, in addition to the various possible chemical and immunological methods for suppression of acquisition of iron by microbial invaders, there remain the more obvious, yet in some cases quite difficult, methods of simply permitting natural host defense mechanisms of nutritional immunity to function. Such methods include avoidance of excessive use of antipyretic drugs, avoidance of excessive dietary and parenteral iron, and attempts to remove underlying causes of either hyperferremia or hypotransferrinemia.

EFFECT OF IRON ON PHAGOCYTOSIS AND IMMUNE RESPONSES

Iron Excess

In addition to its role as a growth stimulant of microbial invaders, excess iron might possibly injure the host by impairing one or more components of either phagocytosis or immune responses. A number of studies have reported that surplus iron alters neither activated neutrophil reduction of tetrazolium salts (9), neutrophil ingestion of bacteria or opsonization by serum factors (9, 32, 37, 77, 106), complement fixation (32), nor immunoglobulin synthesis (37). However, iron has been observed to inhibit the chemotactic (9) and the bactericidal action of leukocytes (77, 78, 194). Staphylococci that had been ingested by rabbit neutrophils remained alive in the presence of 500 μM iron sulfate; moreover, the metal salt neutralized, by precipitation, basic lysosomal proteins that had been released from the host cells. Hemoglobin, myoglobin, and ferritin also were active in proportion to their content of iron; in contrast, hematin compounds with or without iron suppressed killing of bacteria (194). When iron was combined with lactoferrin, its ability to prevent bactericidal action of the lysosomal proteins was enhanced 100-fold; apolactoferrin was inert (194). Observations made on the action of iron sulfate on peroxide (102) suggested that, in addition to precipitation of basic bactericidal proteins, the metal might also impair leukocyte killing by reducing peroxide to harmless water. Furthermore, excess iron also may suppress the bactericidal action of leukocytes by neutralizing their endogenous lactoferrin (34a).

Exogenous siderophores as well as excess iron stimulated mycobacterial growth within phagocytic vacuoles of macrophages derived from sheep monocytes and from mouse peritoneal leukocytes (206). The effect could have been due to a detrimental action of iron on the host cells; however, neither the appearance, maintenance,

TABLE 4. *Conditions in which hyperferremia or hypotransferrinemia have been observed to underlie increased susceptibility of humans to infection*

Condition	Etiology	References
Hyperferremia		
Iron overload	Hemochromatosis and siderosis	(31, 97, 128, 163, 208)
	Neonatal stage (0–30 days)	(12, 20, 57, 175, 191)
	Parenteral iron	(8, 65)
Increased release of iron from stores	Hepatitis	(59, 85, 169)
	Refeeding starvees	(141, 143, 144)
Defective erythropoiesis	Porphyria	(157)
Increased release of iron from erythrocytes	Bartonellosis	(58)
	Malaria	(12, 20, 126, 184)
	Sickling	(7, 12, 20, 27, 176)
	Thalassemia + splenectomy	(41, 64)
	Leukemias, lymphomas, and Hodgkin's disease	(42, 52, 87, 119, 138, 139, 148, 178, 207)
Hypotransferrinemia		
Decreased synthesis of transferrin	Kwashiorkor	(124, 125)
	Jejunoileal bypass	(159)
Insufficient synthesis of transferrin	Neonatal stage (0–30 days)	(12, 20, 57, 175, 191)

metabolism, surface properties, nor permeability functions of the macrophages were altered by the metal. The authors concluded that iron simply was required as a bacterial nutrilite and that "seclusion within tissue cells does not isolate microbes from external nutrients" (206). Stimulation of mycobacterial, brucellar, and plasmodial growth in "inactive" lesions by iron released from stores upon refeeding humans (143) apparently is a clinical example of availability of an extracellular nutrilite for growth of intracellular pathogens. Ferritin iron can be utilized for intra- or extracellular growth of tubercle bacilli, provided that mycobactin is available (108).

Two miscellaneous studies in which excess iron was active might be cited at this point. In one (95), very high molar ratios of iron to toxins were found to inactivate the exotoxin of *Corynebacterium diphtheriae*, the alpha-exotoxin of *Clostridium perfringens*, and endotoxin of *E. coli*. Because of the large metal/toxin ratio, it is unlikely that this phenomenon is physiologically useful in either intoxicated or infected hosts.

In the other study (94), attachment of both piliated and nonpiliated gonococci to human sperm suspended in Ringer solution was enhanced significantly by treatment of the bacteria with 10, but not 1.0, mM iron. Indeed, "gonococci treated with iron adhered to sperm so avidly that they appeared like iron filings attached to a magnet" (94). In contrast, calcium depressed attachment, and magnesium and copper were inactive. The authors proposed that "in the natural disease, there might be substances like iron that help attached gonococci to remain on sperm for possible travel through the female and male urogenital tract." The need for the very large concentration of iron may have been due to the lack of iron-solubilizing agents. If, in fact, physiological quantities are effective in in vivo systems, it would be of great interest to determine whether iron might be involved in attachment of various kinds of microorganisms to a diversity of host cells.

Iron Deficiency

Serum components and various types of leukocytes of human and lower-animal origin have been examined by approximately one dozen laboratories to detect any changes that might be caused by nutritional or hemorrhagic iron deficiency in the host. In those cases in which deficits in the system were observed, restoration of normal iron levels in the host often was sufficient to correct the defect. In general, markedly iron-deficient hosts had greater deficits in phagocytosis and immune responses than did those who were mildly deficient. Adequacy of body iron generally was monitored by Hb or, in some cases, by hematocrit level. In some studies, serum iron and transferrin saturation values also were ascertained in order to confirm that low Hb values were specifically caused by iron deficiency.

In regard to the various components of the process of phagocytosis, opsonization and ingestion of particulate matter by neutrophils remained normal in both mildly and severely iron-deficient children (48, 51, 111); activity of C3 was elevated (122). In rabbits that were made very iron deficient (mean Hb = 5.2 g/dl) by a combination of iron starvation and bleeding, opsonization was normal but ingestion by neutrophils was lowered by 50% (116). The ability of neutrophils to reduce tetrazolium salts in specimens obtained from mildly iron-deficient children was impaired (48, 51) and normal (122).

Intracellular bactericidal action of neutrophils in iron-deficient rats was normal (6). In children, it was normal (111) and depressed (51, 122, 183). In twelve iron-deficient patients, bactericidal activity was depressed in six and normal in six (5). In severely iron-deficient rats, the activity of iron-dependent myeloperoxidase per leukocyte was normal, but the total myeloperoxidase activity per intestine was lowered (6).

Synthesis of immune globulins in iron-deficient children was normal (122, 124, 125) but depressed in iron-deficient rats (114, 146). In humans, tests for measuring cell-mediated immunity such as delayed hypersensitivity, activity of migratory inhibition factor, and T-lymphocyte transformation also have yielded conflicting results. Two groups (82, 111) reported no impairment; five groups (17, 51, 99, 122, 183) observed depression in iron-deficient hosts. In one of the latter studies (183), good correlation of extent of depression with Hb value was observed; for example, mean percentages of formation of rosettes in T cells of children with Hb levels of >12, 10 to 12, 8 to 10, and <8 g/dl, respectively, were 58.7, 54.3, 47.3, and 37.9.

Thus, a few patterns are beginning to emerge concerning the status of nonspecific and immune defenses in the iron-poor host. However, at this stage of our developing knowledge, caution is advised in extrapolation of in vitro laboratory tests to the in vivo situation (50, 73). Recent WHO Technical Reports on nutritional anemias (4, 4a) described the etiological role of hemorrhagic nematodal and trematodal infestation in iron deficiency, but contained only fragmentary information on interactions of iron nutrition with either bacterial, fungal, or protozoan infections. Clearly, well-designed epidemiological studies remain to be performed to ascertain whether mildly and severely iron-deficient persons are more, less, or equally susceptible to infection as compared with iron-sufficient and iron-overloaded hosts. In such studies, the professional team should include not only a hematologist and a nutritionist, but, as important, a microbiologist.

PROSPECTS AND CONCLUSIONS

From the foregoing, it is apparent that many facets of the influence of iron on infection have been identified but that their characterization presently is unequal. Moreover, even with a facet that appears to be fairly well characterized, i.e., host mechanisms of iron withholding during infection, unanswered questions abound. For example, in a summary of a score of reports on human urinary excretion of iron (26), values ranged from approximately 0.2 to 200 μM. There also is considerable fluctuation of urinary iron in the same individual from day to day. Possible correlates of this large variation either with stages of specific metabolic or infectious diseases or, perhaps, with excretion of microbial siderophores should be sought.

Normal 6- to 8-day-old infants were reported to have daily fecal excretion of 1% of their total body iron, whereas their daily intake of iron in human milk was only 10% of the quantity lost (46). Apparently, enhanced fecal excretion of iron is at least one mechanism whereby the healthy neonate can achieve a lowering of its high transferrin saturation. By 2 months, the iron saturation level has been pared to 20 to 30%; despite daily dietary supplements of as much as 36.3 mg of iron (29), it remains at this level throughout the first year of life (191). Other evidence of the efficient homeostatic controls of iron concentration in various body compartments has been obtained by feeding a range of quantities of the metal to various species of lactating mammals. The amount of iron incorporated in milk consistently is observed to be independent of that in the diet (158). In humans, for example, "no relation has been found between the lactating woman's iron intake or Hb concentration and the iron content of her milk" (26).

Nevertheless, despite such efficient homeostatic mechanisms, it is quite probable that spatial and temporal fluctuations of iron availability in a single host organism do occur and that these are sufficient to permit (i) microbial growth in one portion and microbial death in another part of the same tissue, (ii) relapses in which supposedly healed lesions become foci of active infection, and (iii) periodic oscillation in host susceptibility to specific bacterial, fungal, and protozoan pathogens.

Another kind of unanswered question concerns the molecular mechanism(s) whereby LEM and perhaps other hormonal factors are able to override the normal homeostatic controls of host iron metabolism. Moreover, the shifts in iron metabolism that accompany infection and inflammation also are observed in malignancy. Might the host be attempting to combat the growth of tumor cells by iron withholding, or is the shift in iron metabolism simply an aspect of the inflammatory response that is being nonspecifically triggered by the irritant neoplasm?

To what extent can the reproducible and predictable quantitative shift in plasma iron of patients, if monitored daily, be of prognostic aid in infection, inflammation, and malignancy? It may be recalled that in injury of an iron storage tissue, e.g., hepatitis, plasma iron rises rather than falls early in the course of the disease. The return to normal values of plasma iron parallels

and can be used to accurately monitor the rate of liver repair (165). Similarly, the rate of upward return of plasma iron to normal in infection, inflammation, and malignancy parallels the healing of lesions in these conditions. Of course, to avoid diurnal wobble, the sera should be sampled at a constant hour each day.

Still other questions that are just beginning to be considered have been mentioned earlier; for example, to what extent can (and should) the infected host be aided by intensification of the transient lowering of iron (or raising of iron-binding capability) in specific tissues? Possible candidate agents have been suggested: bacterial cell wall products, LEM, lactoferrin, and siderophores of natural or synthetic origin. Might use of these agents especially be appropriate to aid such antibiotics as the tetracyclines that are neutralized by iron (198) or such potentially toxic drugs as the polyenes? Might such agents also be useful adjuncts in tumor cell chemotherapy?

Research also is needed to determine whether siderophores should be considered for incorporation into such products as dentifrices and other prophylactic medications used on the skin and in various nonsystemic tissue sites. Enterochelin was detected in foods that had been exposed to gram-negative bacteria during storage (196), but it is not known to what extent we may be aided or injured by either ingested siderophores or those synthesized in our intestine. If siderophores are developed or discovered that are active against a specific spoilage or pathogenic organism (e.g., *Salmonella* species in smoked meats), these compounds might be useful additives in processed foods.

The much lower incidence of gastroenteritis in breast-fed as compared with formula-fed intants (15, 101, 134) may, to a considerable but unknown extent, be due to maternal lactoferrin plus antibodies (34). Research is needed to determine the amount of iron that can safely be added to the diet of infants without saturating the lactoferrin content of the food. In a thorough review of nutritional factors in anemia (191), Wadsworth stated, " ... in all animals tested, the milk of the species allows an impressive accumulation of iron by the young during the time that they feed solely on milk. ... There is no evidence that during the first few months of life it is necessary to give the baby any more iron than is present in milk." Nonetheless, milk formulas often are supplemented with iron; if this practice continues, should human lactoferrin also be fed to provide some protection to the infant against enteric bacteria while at the same time permitting intestinal absorption of the metal?

In conclusion, during the past half century, research and clinical observations have revealed a vigorous competition for growth-essential iron between bacterial, fungal, and protozoan pathogens and their vertebrate hosts. The latter possess an array of mechanisms (collectively termed nutritional immunity [106]) to withhold iron; the ability of microorganisms to overcome these mechanisms is an important component of virulence. Various aspects of nonspecific and immune defenses are impaired in severely iron-deficient hosts, whereas nutritional immunity is stressed in a diversity of conditions of iron overload. A number of possible methods and agents for suppressing or enhancing various facets of the influence of iron on infection are now apparent. Research is needed to determine which of these might be safe, efficacious, and practical.

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