NEUTROPHIL BACTERICIDAL DYSFUNCTION TOWARDS OXIDANT RADICAL-SENSITIVE
MICROORGANISMS DURING EXPERIMENTAL IRON DEFICIENCY

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I. INTRODUCTION

An adequate supply of iron appears essential to the optimal function of several important cell lines involved in the host's defense against infection. Conversely, during iron deficiency a number of cellular immune dysfunctions have been demonstrated, particularly in regard to polymorphonuclear neutrophils.

In eight of nine studies (1,6,7,8,9,19,28,31,33) of children with iron deficiency neutrophil (PMN) microbicidal activity was reportedly reduced (Table 1). Nitroblue tetrazolium (NBT) reduction by PMNs was found decreased in two studies (6,8) and normal in four investigations (9,12,19,33). Neutrophil myeloperoxidase (MPO) activity was normal in two reports (12,33) and decreased in two others (1,13). Hexose monophosphate shunt activity, tested in one study (33), was found to be defective. These available data, although fragmented and incomplete, suggest the existence of a defect in the bactericidal oxidative machinery of neutrophils, which could affect the development of clinical infections in some patients with iron deficiency (for a recent review of the much debated relationship between infections and iron balance, see ref 15).

Studies of PMN function in ID remain controversial for several reasons. First, in at least five of these studies, the neutrophil dysfunctions shown were present in association with nutritional deficiencies other than iron (1,6,8,9,19). Such nutritional deficiencies, especially protein-calorie malnutrition, can by themselves lead to PMN microbicidal defects. Secondly, some of the patients studied had concurrent or recent infections (8,19), which can also alter the functions of PMNs and their metabolism.

The purpose of this research is to clarify the contribution of isolated nutritional iron deficiency to neutrophil dysfunctions in experimental animals, in the absence of other nutritional deficiencies and of infections. Our specific objectives are to establish a proper animal model for the development of pure iron deficiency without attendant malnutrition; to determine whether pure iron deficiency causes dysfunction of neutrophil phagocytosis and bactericidal activity; to determine further whether such neutrophil dysfunctions, if present, are associated with defects of the two major iron-dependent enzymes (myeloperoxidase and NADPH-oxidase); and to determine whether correction of the iron deficiency would correct the putative functional and metabolic neutrophil defects.

II. MATERIALS AND METHODS

a. Animals and study design

Pregnant rats (Sprague Dawley, Madison, Wisconsin) were maintained on a standard breeder diet (Teklad, Madison, Wisconsin) until their delivered pups were two weeks of age. At that time the dams were given a low iron diet (ICN Pharmaceuticals, Cleveland, Ohio) containing 8 mg of iron per kg of diet.

Iron deficient rats were compared with iron-sufficient controls. All growing rats received the low-iron diet, which was started at weaning, day 21 after birth. The animals in the iron deficient group received the diet ad libitum, with no iron supplement until neutrophil function studies were completed. The animals in the control group also received the diet ad libitum, but received an intramuscular iron dextran complex diluted in sterile saline to yield individual doses of elemental iron (7.5 mg) at weaning and every two weeks.

Growing rats were weighed regularly at the intervals shown on the study calendar (Fig. 1).

b. Microorganisms, opsonins

A stock culture of Staphylococcus aureus 502A was prepared every 14 days for the duration of the bactericidal assays by streaking onto trypticase soy agar from frozen-thawed individual samples. As working culture, one colony of Staphylococcus aureus was picked from the stock culture, inoculated into trypticase soy broth and incubated at 37°C for 16-18 hours. The bacteria were centrifuged from suspension, rinsed, counted, opsonized and adjusted to equal 20 bacteria per adherent neutrophil (see below).

A frozen culture of smooth Salmonella typhimurium (provided by Dr. Roger Cunningham at SUNYAB) was plated onto trypticase soy agar, and incubated at 37°C for 16-18 hours. The bacterial culture was transferred every 48 hours until it reverted to a rough form after 14 days. A stock culture was then prepared on an agar plate, stored at 5°C and transferred every two weeks. The working culture was processed as described for Staphylococcus aureus.

A stock culture of Streptococcus pneumoniae (mucoid, type III) recovered from a patient sample was propagated in brain-heart infusion medium for three days, cultured and transferred on blood agar plates until it reverted to a rough form, and stored at 5°C on blood agar plates; it was processed as a working culture similarly to the Staphylococcus aureus.

Candida albicans was grown on trypticase soy agar and stored at 5°C. This stock culture was transferred every 30 days. The working culture consisted of one colony of C. albicans from the stock culture inoculated into trypticase soy broth, grown at 37°C for 4 days, opsonized and diluted to equal 10 yeasts per adherent neutrophil.

The viability of opsonized microbial suspensions was tested with acridine orange by fluorescence microscopy (24). The opsonin source in all tests consisted of pooled, inactivated rat serum at a final concentration of 10%, obtained from normal adult, iron-sufficient rats.

c. Hematological values

Hemoglobin levels were determined by the cyanmethemoglobin method. White cell counts were measured with an electronic cell counter (Coulter Electronics, Hialeah, Florida). Percentages of neutrophils, lymphocytes, and monocytes were determined microscopically with routine differential stains. ZPP (zinc protoporphyrin) levels were read on a hematofluorometer (AVIV Associates, Lakewood, N.J.) (18).

d. Neutrophil monolayers and functional tests

Fifty to 100 ul of peripheral blood (tail vein) was allowed to clot on glass coverslips at 37°C. The clots were rinsed off and the adherent cells counted under the microscope in 10 high-power fields per coverslip. More than 99% of these cells were neutrophils. The approximate number of neutrophils per coverslip was determined (usually 1.02×10^5), and microbial suspensions were adjusted to equal 20 bacteria and 10 fungi per neutrophil. Bacterial suspensions were thus diluted to 2.0×10^6 bacteria/ml and 100 ul amounts were applied to the coverslips. Yeast suspensions were diluted to equal 1.0×10^6 yeast/ml and 100 ul amounts were applied to the coverslips. Careful evaluation of PMN adhesion to the coverslips during three different sets of paired experiments revealed no difference in adhesion between the two groups of animals (data not shown).

A modification of the supravital fluorescent method of Pantazis was used to determine ingestion and killing of all microorganisms (24). After 1 hour of PMN/microorganism interaction, the monolayer was rinsed free of its microorganism suspension and stained with a 0.003% acridine orange solution. The monolayers were examined by fluorescence microscopy for dead microorganisms (red fluorescence) and viable organisms (green fluorescence).

Phagocytosis was determined by counting the number of red and green organisms present in the neutrophils, and was reported as numbers of phagocytized organisms per 100 PMNs (phagocytic capacity) (29). Viability of PMNs was also determined by evaluation of fluorescence patterns (red vs green).

e. Metabolic assays

Myeloperoxidase determination was a modification of the semi-quantitative method of Cech (4). Briefly, fresh films of peripheral blood were fixed and treated with benzidine and hydrogen peroxide. The oxidized benzidine precipitates as dark blue granules in the PMN cytoplasm at a pH of 6.0 only in the presence of MPO at the exclusion of catalases. Smears of counterstained cells, examined by light microscopy were given a histochemical score of 0 to 3+, based upon the density of the blue deposits. Total scores were determined from the analysis of 100 neutrophils.

Stimulated neutrophil NBT reduction was assayed by a modification of the methods of Humbert and Park (14, 25). Phorbol myristate acetate (PMA) was used to stimulate (32) the cells which at completion of the test, were deposited onto glass slides by centrifugation. The stained cells were evaluated semi-quantitatively for formazan content with a scoring system of 0 to 3+, as described for myeloperoxidase activity. All histochemical tests were read in blind fashion with the observer unaware of samples' belonging to ID or control groups.

F. Statistical Analysis

Both the paired t-test and the Wilcoxon-Mann-Whitney rank-sum analysis were used in data analysis. Significance was defined as a p value of < 0.05 by both statistical analyses.

IV. RESULTS

Weight, Hematological values

The results in 20 animals raised for this study are summarized on Fig. 1. Between day 32 and 94, the ten iron-deficient animals had significantly lower hemoglobin concentrations than their controls; hemoglobins dropped to a mean of 6-6.5 g/dl between days 49 and 86 of the study (control values - 13.5 to 14.0) during which time all functional and metabolic studies were done. That the hemoglobin drop was due to iron-deficiency is documented by a significant increase in the ZPP values of iron-deficient rats during the same period. Upon completion of the neutrophil function studies, iron deficiency was repaired in deficient animals by administration of Imferon on day 101; the hemoglobin in these rats rose by day 154 to an average of 14.2 grams and the ZPP levels dropped to normal values. Leukocyte counts from both groups of animals were essentially identical at each of the six study periods (from day 23 to day 154) during which they were evaluated (data not shown).

Bactericidal and candidacidal activity

During iron deficiency, bactericidal activity towards S. aureus was significantly decreased in iron deficient animals (Fig. 2). These animals killed only 29 percent of the ingested S. aureus vs 72% for the control animals (p < 0.0025). There was no difference in bactericidal activity towards S. pneumoniae between the iron deficient animals (35.3 percent bacterial killed) and the control animals (36.9 percent bacteria killed). Microbicidal activity against S. typhimurium was equal in the iron deficient and iron sufficient animals (control animals 26 percent, iron deficient animals 26.7 percent).

Candidacidal activity was significantly depressed in iron-deficient animals (31.7 percent candida killed vs 60.7 percent killed by controls, p < 0.005) and returned to normal values on day 150 after correction of the iron deficiency (Fig. 3), when PMNs of both sets of animals killed approximately 60 percent of the candida ingested.

Phagocytosis

Phagocytic activity was unaffected by iron deficiency when evaluated with Salmonella typhimurium, Staphylococcus aureus, and Streptococcus pneumoniae (Table 2). Phagocytic activity towards Candida albicans showed a small but significant difference during the iron deficiency period, which normalized after the iron status was corrected (Table 4).

NBT reduction, MPO activity

NBT reduction was profoundly decreased (by 50-60%) in PMNs of iron-deficient rats following stimulation of PMNs with phorbol myristate acetate. After iron reconstitution of the deficient animals, NBT reduction returned to normal (Fig. 4, Table 4). Myeloperoxidase activity was moderately, but significantly decreased in iron-deficient rats and returned to normal after the iron deficiency was corrected (Table 5).

DISCUSSION

Our results in rats are compatible with those of several other investigators who have shown decreased bactericidal capacity during iron deficiency in humans. Chandra's studies, like ours, excluded protein-calorie malnutrition as a cause of the defect, although his subjects could have had other nutritional deficiencies. In three of his reports, intracellular killing of Staphylococcus aureus was reduced in iron-deficient children and normalized following iron therapy (6,7,8). In one of his studies, the defect was greater in children with moderate iron deficiency than in the severely iron deficient children (8). He proposed two explanations for this apparent paradox; first, that the bactericidal defect was already at a maximum by the time the patients reached moderate iron deficiency; secondly, that there was some iron available in the neutrophils of moderately iron deficient patients, enough iron to support intracellular bacterial growth. The same author also found that when neutrophils of obese children had reduced bactericidal activity, these values correlated only with co-existing iron deficiency.

Several other studies of human PMNs, within the limitations expressed earlier, have further suggested a bactericidal defect towards Staphylococcus aureus (see Table 1). In iron deficient rats who had no other nutritional defect and no infection we have now established the existence of a distinct bactericidal defect. Our findings strongly suggest that in the previously reported human studies, the bactericidal defect was due at least in part to iron deficiency, rather than to the co-existing malnutrition and/or infections. This conclusion is further supported by the degree of decrease in bactericidal activity reported by others which is very similar to that observed in our study, i.e., a 30 to 35% derivation from normal values.

The killing of Candida albicans by iron deficient rat PMNs was affected in the same degree as that of Staphylococcus aureus: only 31 percent of the candida were killed by the iron deficient animals while the controls were able to kill approximately 60% of the organisms ingested (Table 3, Fig. 3). This candidacidal defect, not previously described in iron deficiency, has a clinical counterpart in two studies of chronic mucocutaneous candidiasis occuring in children with iron deficiency (12): Higgs' patients, when treated with iron, showed definite clinical improvement of their candida infection. When our rats were restored to iron sufficiency, their neutrophils killed candida normally (Table 3, Figure 3). Evaluated together, these data suggest that the PMN cidal defect consequent to iron deficiency may play an important role in the perpetuation of candida infections, especially in patients suffering from associated immune deficiencies.

When we investigated killing of Streptococcus pneumoniae, a catalase-negative bacterium, we found no differences in bactericidal activities between the two groups of animals (Fig. 2). There are no other published studies of PMN bactericidal activity towards Streptococcus pneumoniae during iron deficiency. These findings led us to analyze the biochemical basis for this interesting differential cidal defect.

Bacteria are killed mostly by oxidant radicals (superoxide, hydrogen peroxide, and hypochlorite), products of the neutrophils' oxidative pathway (17,26). Basically, following the phagocytic stimulus superoxide is produced by a membrane-bound NADPH-oxidase (10). In the phagocytic vacuole, superoxide is converted to H_2O_2, a moderately bactericidal substance (26). H_2O_2 then combines with myeloperoxidase, discharged from the azurophilic granules which have fused with the phagosome; the H_2O_2:MPO complex can oxidize compounds which donate electrons such as the halides. Iodination, bromination, or chlorination of the bacterium can occur through the substitution of a halide for a hydrogen at a crucial bacterial site (17).

Many bacteria and yeast also produce H_2O_2, which is disposed of, in most cases, by bacterial catalase (20). There are major differences in the killing of catalase-positive and catalase-negative organisms by neutrophils. To be killed effectively by neutrophils, catalase-positive organisms such as Staphylococcus aureus and Candida albicans must be attacked by PMN-originating H_2O_2 accompanied by the peroxidase-halide system (26). If a neutrophil cannot generate superoxide (and therefore, H_2O_2) it cannot efficiently kill catalase-positive organisms (17). Catalase-negative bacteria, such as pneumococci produce enough H_2O_2 to undergo self destruction in the presence of MPO and halides after phagocytosis by PMNs, even if the cell lacks the capacity to produce H_2O_2(17).

If neutrophil enzymatic activity for NADPH oxidase is defective, this cell will not generate superoxide and in turn will produce no H_2O_2; it will not kill catalase-positive organisms but will kill catalase-negative organisms normally. This is the case in chronic granulomatous disease (17). When there is simply a decreased level of NADPH oxidase, as in many CGD-carrier states, where half the neutrophils are normal and half lack NADPH-oxidase, bactericidal activity towards catalase-positive bacteria is approximately 50% of normal (21).

In our study, the iron deficient animals' neutrophils displayed a decrease in their ability to kill Staphylococcus aureus and Candida albicans (40 and 52 percent of normal values, respectively, Figs 2 and 3), reminiscent of the situation existing in the chronic granulomatous disease carrier state. As in this disorder, no defect in the killing of the catalase-negative pneumococcal organisms is seen (Fig. 2). We therefore suspected a defect in the oxidative metabolism of neutrophils in our iron deficient animals and investigated the production of oxidant radicals with a semi-quantitative histochemical NBT reduction test.

NBT dye is an artificial acceptor which is reduced to a visible blue dye by the PMN during the phagocytic-induced metabolic burst; the reduction is due, under PMA stimulation, to the direct action of superoxide produced by NADPH-oxidase (32). NBT reduction in our iron deficient animals was about 1/2 of that in the control animals, both in resting and stimulated neutrophils, suggesting a defect in NADPH-oxidase activity as the basis for the pattern of bactericidal-candidacidal activity (Table 4, Fig 4).

Three other studies have reported reduced NBT in iron deficiency. Chandra (6,8) reported decreased levels in iron deficient patients using the quantitative NBT test. In one study of rabbits made iron deficient by repeated bleeding, the mean resting value for NBT reduction (histochemical test) was reduced to 10% of the control value (5). Four groups of investigators, however, reported normal or elevated NBT-reduction values in iron deficient children (9,12,19,33); in these studies at least some of the children were infected. Infection increases NBT-reduction activity by PMNS (14) and this circumstance could account for the normal to elevated values observed by these authors. In our study, NBT-reduction values returned to normal after the animals had adequate iron levels (Table 4, Fig. 4). Similar normalization of low NBT-reduction values during iron deficiency were observed in Chandra's human studies, when iron nutriture was corrected (6,8). NADPH-oxidase is a coupled enzyme, composed of several moieties, one of which is an iron-containing and iron-dependent cytochrome-b (3); we suggest that

iron deficiency could alter the function of this enzyme, with a consequent pattern of bactericidal and fungicidal defects similar to that of some cases of CGD (10,27). These defects are completely correctable when there is a return to adequate iron status.

Similarly to our finding of decreased bactericidal activity towards Staphylococcus aureus and Candida albicans in the neutrophils of iron deficient children, Srikantia found that neutrophils from iron deficient children killed poorly Escherichia coli, a catalase-positive Gram-negative organism. The bactericidal activity of these leukocytes correlated with the decrease in hemoglobin levels, and was significantly depressed when hemoglobin levels fell to 10 g/dl or less. We therefore expected to find a decrease in the killing of Salmonella typhimurium, another catalase-positive Gram-negative bacterium. We found, however, no differences between iron deficient and control neutrophils in their ability to kill Salmonella typhimurium (Table 2). Salmonella typhimurium is characterized by an unusually long intracellular survival, possibly in part because of the length of the lipopolysaccharide structure (23). This peculiar intracellular survival may have masked possible differences in ultimate killing ability by PMNs because of the relatively short incubation time used in our experiments. Indeed, the rate of killing of salmonella was the lowest of all organisms studied, with both iron deficient and normal PMNs (about 26%, see Fig. 2), and a longer incubation may have revealed an ultimate bactericidal difference. It is also possible that in our cellular model the salmonella organisms were killed by non-oxidative bactericidal mechanisms, as suggested by Okamura and Spitznagel (23). Those authors compared the respective bactericidal activity by oxygen dependent and independent mechanisms of human PMNs challenged with Salmonella typhimurium LT2 and its outer membrane mutants. Anaerobic conditions were established so that the neutrophils exhibited no superoxide production and could not reduce NBT. Yet these same neutrophils killed the salmonella in an orderly fashion that appeared to be dependent on their lipopolysaccharide structure.

Most studies of myeloperoxidase determined by histochemical technique measure only the absence or presence of MPO; however one other report of MPO activity determined by histochemical score was compared to a quantitative biochemical assay (4). The two methods were shown to correlate favorably where complete, incomplete, and missing enzyme were demonstrated in a large family with MPO deficiency. We therefore felt confident to quantify rat MPO activity with a histochemical score method, since the small amounts of blood cells available precluded the use of quantitative enzyme assays.

PMN myeloperoxidase, showed a small, but significant decrease in the cells of the iron deficient animals. This measurement occurred during a 7-week period of iron deficiency which coincided with rapid growth (Table 5). While several authors suggest a decrease in MPO levels during human iron deficiency others hesitate to link these two deficiencies. Our scoring method indicates that MPO is moderately decreased in an iron deficient model uncomplicated by other nutrient abnormalities.

We also report the noramlization of cellular MPO scores after correction of the iron deficiency in our animals (Table 5). Arbeter (1) also found decreased MPO levels in his iron deficient subjects which corrected after the iron levels returned to normal. An interesting finding in his study is that two patients treated for kwashiorkor and malnutrition developed MPO deficiency, while they also developed transient iron deficiency. Their MPO levels corrected as their iron levels were normalized.
The relatively small decrease in MPO activity cannot account for the major defect in the staphylocidal activity already discussed. Klebanoff reviewed the staphylocidal activity of normal and MPO-deficient PMNs (17). In MPO-deficient PMNs, bacteria were killed after a lag period which indicates that MPO is involved early after phagocytosis and that other killing systems which operate more slowly remain intact. In our study, the significant impairment in the killing of Staphylococcus aureus probably reflected the serious defect in NADPH oxidase. On the other hand, candidacidal activity may be exquisitely sensitive to the levels of MPO (17). Whether the modest MPO deficiency we observed may have contributed in part to the abnormality in destruction of that fungus is somewhat doubtful, however.

MPO activity (Table 5) decreased slightly (but significantly) from the initial set of normal values determined at 93 days, and the final set of values measured at age 154 days. These changes probably reflect maturational variations in the growing rat; such adaptive changes have been described in human infants for neutrophils NBT-reductase and PMN glucose-6-phosphate dehydrogenase (2).

The possibility that parenteral iron could have stimulated PMN functions was dismissed for several reasons. Firstly, the differences in PMN functions between ID and control animals was selective to two organisms rather than general, as would have been expected from an overall immune enhancement. Secondly, microbicidal activity of ID animals was comparable to that of controls after correction of ID with a single injection of iron some two months earlier; such a long interval seems incompatible with the stimulation of cells as short-lived as PMNs. Finally, normal values were within the range of those observed for other animals with iron sufficiency due to normal diets, who were studied in early experiments while we were establishing the assays (data not shown).

Phagocytosis of three bacteria, Staphylococcus aureus, Streptococcus pneumoniae, and Salmonella typhimurium was normal in iron deficient rats, before and after iron repletion. We did find a lowered phagocytic capacity to Candida albicans in the iron deficient animals, which corrected after administration of iron (Table 3). This defect has never been reported previously. Other investigators of PMN phagocytosis during iron deficiency have used Staphylococcus aureus and Escherichia coli as test organisms and have reported normal ingestion of these organisms, as we also have for Staphylococcus aureus.

Our explanation for the lack of a generalized defect in phagocytosis during iron deficiency may be that the energy producing and structural mechanisms involved in ingestion are not altered in response to low iron levels. Engulfment involves the assembly of microfilaments made of polarized contractile proteins (30). The assembly of these proteins to produce vacuole invagination, closure, and also degranulation is related to the release of calcium ions from the plasma membrane and to the stimulation and control exerted on the PMN by cGMP and cAMP, respectively (16). None of these processes depends on iron for function.

Phagocytosis of a foreign particle begins when the PMN recognizes it by virtue of its opsonin (11). Most opsonins are either antibodies (IgG) that are keyed to the surface of the particle or derivatives of the complement system. Decreased numbers of antibody forming cells and complement alterations have been described in iron deficiency (22). However, we used normal (iron-sufficient) rat serum opsonins with the bacteria studied and therefore cannot attribute the specific candida phagocytic defect of iron

deficient rats to a humoral factor. The cellular phagocytic defect towards Candida albicans remains unexplained at present, although it suggests the existence of a reversible, iron-dependent candida-specific neutrophil membrane defect.

IV. SUMMARY AND CONCLUSIONS

We developed a clear-cut nutritional iron deficiency anemia without concomittant malnutrition in rats given a low iron diet, and we restored normal iron and hemoglobin levels in these same animals with iron dextran injections.   The neutrophil function studies performed during and after a period of iron deficiency showed the following:
1. Phagocytosis of Staphylococcus aureus 502A, Streptococcus pneumoniae, and Salmonella typhimurium was not altered by iron deficiency or by the administration of iron; phagocytosis of Candida albicans was moderately abnormal during iron deficiency, and became normal with the restoration of iron sufficiency.
2. Microbicidal activity towards Staphylococcus aureus 502A and Candida albicans, two catalase-positive microorganisms, was markedly decreased (to 50% of control values) and returned to normal when iron sufficiency was restored. Killing of a catalase-negative organism, Streptococcus pneumoniae was normal in iron-deficient rats. This pattern of differential bactericidal activities suggested an abnormality of the oxidant radical-generating machinery in neutrophils of iron-deficient animals.
3. Indeed, iron deficiency caused a marked decrease of neutrophil nitroblue tetrazolium dye reduction, which disappeared after iron administration.
4. Neutrophil myeloperoxidase activity was slightly decreased in iron deficient rats and returned to normal after iron administration.
5. Microbicidal activity towards a gram-negative, catalase-positive organism, Salmonella typhimurium, was equal in iron deficient and iron sufficient animals.

Our combined results suggest that a definite microbicidal defect is the consequence of nutritional iron deficiency, apart from any protein-calorie malnutrition. This defect affects the disposal in PMNs of two catalase-positive microorganisms (which require intracellular production of oxidant radicals for their destruction) but not of a catalase-negative bacterial species. As evidenced by the transient defect in NBT-reduction, iron deficiency causes a pronounced impairment of PMN superoxide production, which can fully account for the transient, selective microbicidal defect observed. Consequently, iron deficiency produces in PMNs, a defect similar to that of certain patients with chronic granulomatous disease (although, less pronounced) because of the alteration of the same critical iron-containing enzyme, NADPH-oxidase, which is responsible for superoxide production. Whether this enzymatic impairment is due to a reduction or dysfunction of the iron-containing cytochrome-b moiety of NADPH-oxidase is conjectural at this time. Another iron-containing microbicidal enzyme, MPO, is moderately altered, and may minimally contribute to the staphylocidal and candidacidal defect observed.

The lack of an observed bactericidal defect towards salmonella species, during iron deficiency, could relate to the relatively short experimental intracellular exposure for that fastidious organism, or to its sensitivity to non-oxidative bactericidal mechanisms. The unexpected dysfunction of PMN phagocytosis observed in regard to Candida albicans suggests a specific iron deficiency-dependent, reversible membrane defect of neutrophils. Taken together, these PMN dysfunctions could account for the increased susceptibility towards bacterial and fungal infections observed in some subjects with iron deficiency.

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TABLE 1. SUMMARY OF NEUTROPHIL FUNCTION STUDIES IN IRON DEFICIENT PATIENTS

AUTHOR	REFERENCE	PHAGOCYTOSIS	BACTERICIDAL ACTIVITY	NBT REDUCTION	MPO	COMMENTS
Arbeter	1	–	↓	–	↓	malnutrition
Higgs	12	–	–	normal	normal	infection
Chandra	6	normal	↓	↓	–	malnutrition
Chandra	8	normal	↓	↓	–	infection, malnutrition
McDougall	19	–	↓	normal	–	infection, malnutrition
Srikantia	28	–	↓	–	–	malnutrition
Suskind	31	–	normal	–	–	parasite infection
Chandra	7	normal	↓	–	–	zinc deficiency
Yetgin	33	normal	↓	normal	normal	malnutrition
Foroozanfar	9	normal	↓*	normal	–	malnutrition

↓ = decreased

↑ = elevated

– = not done

MPO = myeloperoxidase activity

* = Candidacidal activity

TABLE 2. NORMAL NEUTROPHIL PHAGOCYTIC CAPACITY TOWARDS THREE MICRO-ORGANISMS (organisms/neutrophil)

Test Organism (day of test)	S. typhimurium (day 51)	S. aureus (day 78)	S. pneumoniae (day 73)
Iron Deficient Animals (10)	8.15	7.05	6.2
Control Animals (10)	7.71	7.78	6.5
SD	1.0	0.7	0.6
P	NS	NS	NS

TABLE 3. PHAGOCYTIC CAPACITY TOWARDS CANDIDA ALBICANS DURING IRON DEFICIENCY AND AFTER ITS CORRECTION (organisms per neutrophil)

(day of test)	Before Iron (day 64)	After Iron (day 152)	SD	P
Iron Deficient Animals (10)	4.5	5.1	0.6	<0.025
Control Animals (10)	5.3	5.1	0.3	NS
SD	0.6	0.3		
P	< 0.005	NS		

TABLE 4. DECREASED NITROBLUE TETRAZOLIUM REDUCTION DURING IRON DEFICIENCY AND AFTER ITS CORRECTION (phorbol myristate acetate-stimulated neutrophils, histochemical score)

	Before Iron (day 93)	After Iron (day 154)	SD	P
Iron Deficient Rats	112	239	27.1	< 0.0025
Control Rats	252	237	31.4	NS
SD	42.3	13.4		
P	< 0.0025	NS		

TABLE 5. NEUTROPHIL MYELOPEROXIDASE ACTIVITY DURING IRON DEFICIENCY AND AFTER ITS CORRECTION (histochemical score)

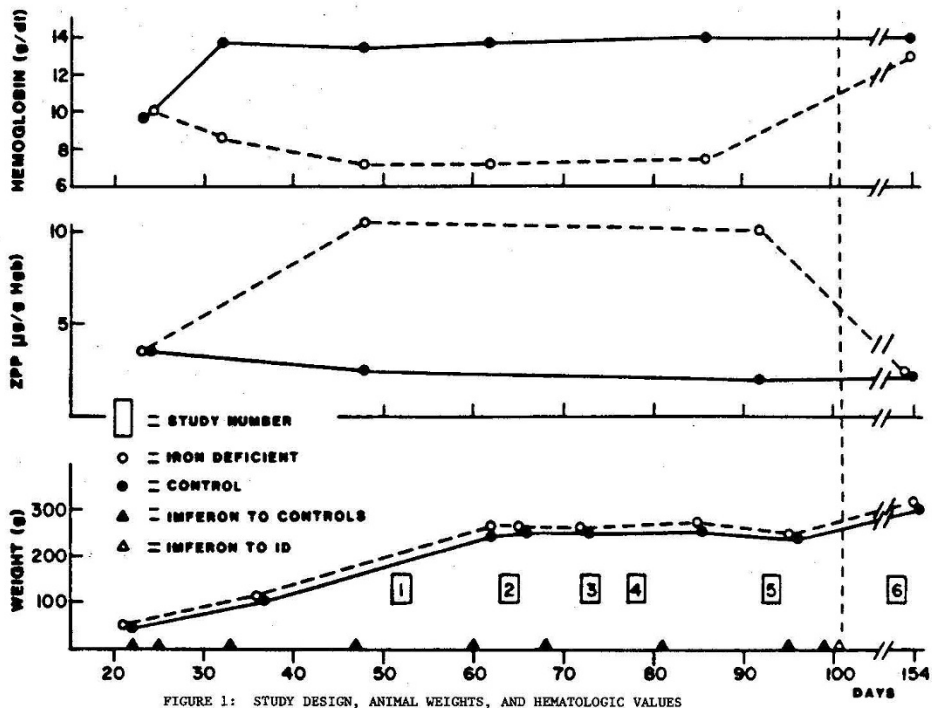| (day of test)	Before Iron (day 93)	After Iron (day 154)	SD	P
Iron Deficient Rats (10)	243	266	22.5	< 0.01
Control Rats (10)	271	265	7.9	< 0.05
SD	21.6	7.5		
P	< 0.005	NS		

FIGURE 1: STUDY DESIGN, ANIMAL WEIGHTS, AND HEMATOLOGIC VALUES

Development of anemia in nutritional iron deficient growing rats and correction of anemia after iron repletion is shown here. Boxes with numbers refer to individual test periods during which the following neutrophil function tests were performed: 1 = Bactericidal activity against Staphylococcus aureus, 2 = Candidacidal activity, 3 = Bactericidal activity against Streptococcus pneumoniae, 4 = Bactericidal activity against Salmonella typhimurium, 5 = Nitroblue tetrazolium reduction, myeloperoxidase activity, 6 = Candidacidal activity, nitroblue tetrazolium reduction, and myeloperoxidase activity. Results of these neutrophil function tests are summarized in Figures 1-4 and Tables 2-5. The lower third of the figure demonstrates the lack of weight difference between iron deficient and control animals. The remainder of the graph illustrates the profound anemia, accompanied by elevated erythrocyte zinc protoporphyrin (ZPP) levels, which developed in the iron deficient animals. Hemoglobin and ZPP levels returned to normal after correction of the iron deficiency on day 100.
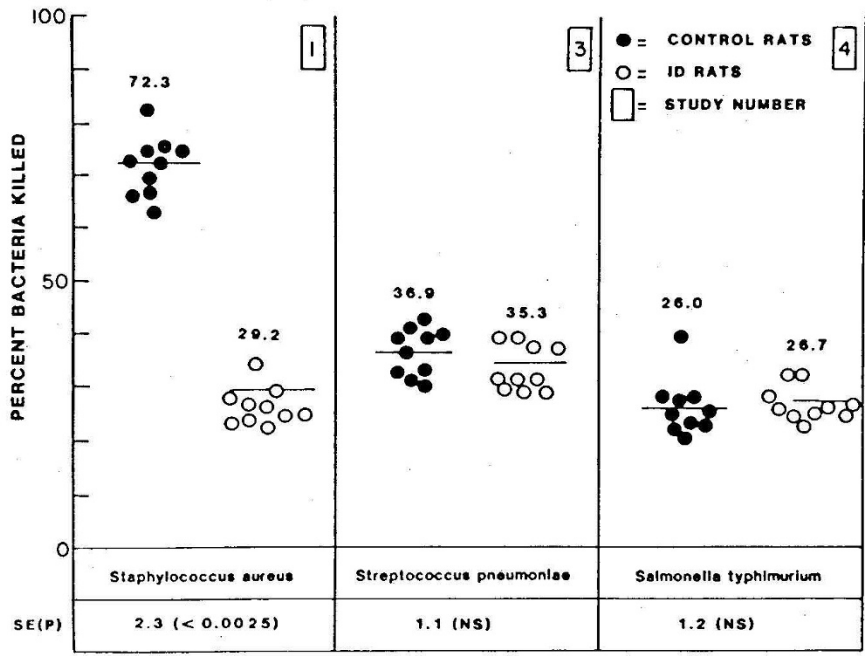

FIGURE 2: NEUTROPHIL BACTERICIDAL ACTIVITY TOWARDS 3 ORGANISMS IN IRON DEFICIENT RATS

Numbers in graph = mean values. The boxed "study number" refers to the individual test periods; 1 = tests performed at 52 days of age, 3 = tests performed at 75 days of age, and 4 = tests performed at 79 days of age (see Figure 1). There is a significant decrease of neutrophil bactericidal activity towards Staphylococcus aureus 502 A in iron deficient animals. Streptococcus pneumoniae and Salmonella typhimurium are killed normally by iron deficient neutrophils.
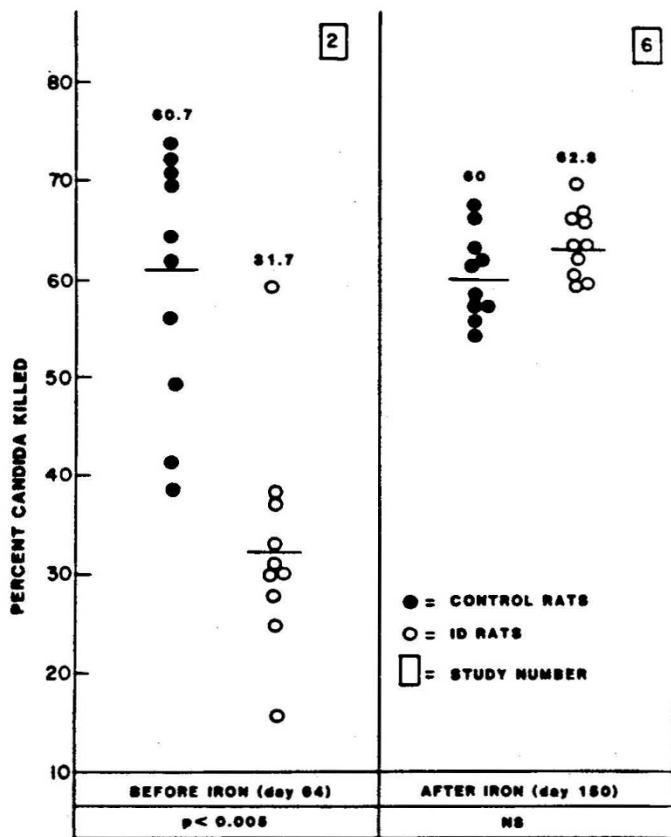
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FIGURE 3: NEUTROPHIL CANDIDACIDAL ACTIVITY DURING IRON DEFICIENCY AND AFTER ITS CORRECTION

The significantly decreased candidacidal activity of iron deficient neutrophils normalizes after iron repletion. The boxed numbers correspond to the test periods indicated on Fig. 1. Horizontal bars = mean values.
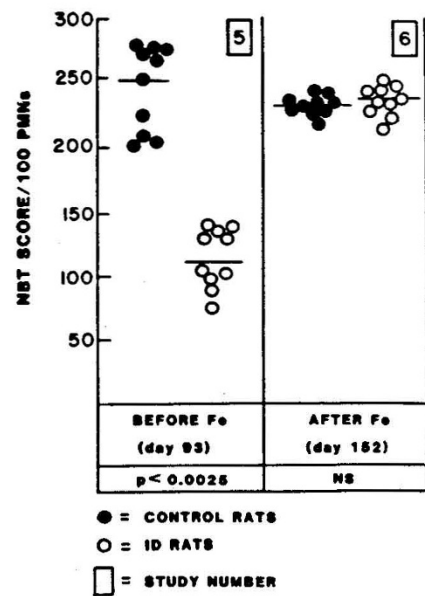

FIGURE 4: NITROBLUE TETRAZOLIUM REDUCTION IN PHORBOL MYRISTATE ACETATE-STIMULATED NEUTROPHILS

In rat neutrophils, iron deficiency (ID) induces a pronounced decrease of NBT reduction, which returns to normal after correction of the iron deficiency. (Fe = administration of iron)