Review

Natural resistance, iron and infection: a challenge for clinical medicine

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Natural resistance to infection, which does not depend on antibiotics, is a powerful protective mechanism common to all mankind that has been responsible for the survival of our species during countless millennia in the past. The normal functioning of this complex system of phagocytic cells and tissue fluids is entirely dependent on an extremely low level of free ionic iron (10^{-18} M) in tissue fluids. This low-iron environment is maintained by the unsaturated iron-binding proteins transferrin and lactoferrin, which depend on well-oxygenated tissues, where a relatively high oxidation–reduction potential (Eh) and pH are essential for the binding of ferric iron. Freely available iron is derived from iron overload, free haem compounds, or hypoxia in injured tissue leading to a fall in Eh and pH. This can severely damage or abolish normal bactericidal mechanisms in tissue fluids leading to overwhelming growth of bacteria or fungi. The challenge for clinical medicine is to reduce or eliminate the presence of freely available iron in clinical disease. In injured or hypoxic tissue, treatment with hyperbaric oxygen might prove very useful by increasing tissue oxygenation and restoring normal bactericidal mechanisms in tissue fluids, which would be of huge benefit to the patient.

Introduction

The rapid increase in bacterial resistance to antibiotics has resulted in increased morbidity and mortality among patients in hospitals, and in intensive care units in particular. Various measures have been proposed for alleviating this situation, such as increased surveillance, improved physical controls like hand washing, and the substitution of empirically employed broad-spectrum antibiotics by narrow-spectrum drugs to which the infecting organism is known to be sensitive (Vincent *et al.*, 2004). These and other measures can be used as a concerted attempt to stop a process that yearly has been getting worse. However, experience suggests that bacterial resistance to antibiotics will continue to increase for as long as the use of antibiotics provides the selective pressure (National Institute of Allergy and Infectious Diseases, 2004; Murray, 2000). In that case what is to be done? Is there any system of infection control that does not involve antibiotics? The answer is yes, and this system has been with us for a very long time.

Natural resistance to infection has been responsible for the survival of our species, in spite of repeated attacks by bacteria. Infections like bubonic plague, syphilis, tuberculosis, cholera, and many others, have resulted in many casualties, but always there have been survivors capable of passing on their mechanisms of resistance to future generations.

The protective systems of natural resistance need to be exploited in various different ways, but one single fact is

paramount: the ability of the complex system of phagocytic cells and tissue fluids to resist bacterial invasion depends entirely on an extremely low level of free ionic iron (about 10^{-18} M) in tissue fluids such as plasma. This low-iron environment is due to the iron-binding proteins transferrin and lactoferrin, which are normally only 30–40 % saturated with iron and have very high association constants for ferric iron of about 10^{36} (Bullen *et al.*, 1978, 2005). Freely available iron can severely damage or destroy the whole mechanism of natural resistance, leading to rapid bacterial or fungal growth in tissue fluids. In addition, it is essential that normal physiological conditions in tissue fluids, such as the pO_2, pH and the oxidation–reduction potential (Eh), are taken into account since these physical conditions are closely involved in controlling the availability of iron for bacteria (Bullen *et al.*, 2005).

There are three main sources of free iron during clinical infections. First, iron overload due to altered metabolism, such as during haemochromatosis and hepatic disease, cancer or following chemotherapy. Second, iron from free haem compounds following the lysis of red cells. Third, iron made available from normal fluids and tissues as a result of trauma and altered physiological conditions.

There are ample data to show that the availability of iron can and does play a critical role in many different clinical infections. This represents a challenge for clinical medicine to investigate the role of iron availability more closely. At

present there appears to be a general unawareness of the relevance of this topic for clinical medicine, and its close involvement in bacterial invasion and host resistance. Continuing problems with antibiotics make it more urgent than ever to explore new methods of enhancing natural resistance. If successful this could be of great benefit, not least because no antibiotics are involved.

Infections with iron overload

Haemochromatosis and hepatic disease. Patients with haemochromatosis or severe hepatic disease have abnormal levels of stored iron. Normally persons possess 1–2 g of iron. In haemochromatosis the range is 8–37 g, much of it stored in the liver. In these patients there is often an abnormally high level of saturation of serum transferrin with iron (McLaren et al., 1983), and there may also be a pool of freely available low molecular mass iron in the plasma, detectable by the bleomycin assay, that is not present in normal persons (Gutteridge et al., 1985).

Patients with haemochromatosis and other syndromes involving iron overload are unusually susceptible to *Vibrio vulnificus* infections. This organism lacks an efficient high-affinity iron-uptake system, and cannot grow in inactivated (complement free) normal human serum (Chart & Griffiths, 1985) It is easily destroyed by fresh human plasma, but multiplies rapidly in the plasma of haemochromatosis patients with high levels of freely available iron (Bullen et al., 1991).

V. vulnificus septicaemia is one of the most striking examples of enhanced susceptibility to infection. It begins abruptly without any apparent focus of infection and often follows the eating of raw sea food such as oysters. Of 24 patients with primary septicaemia, 18 had pre-existing hepatic disease, including haemochromatosis, chronic cirrhosis and hepatitis. Of the remaining six, three had a history of alcoholism, one of thalassaemia, one had diabetes, and one had no underlying disease (Blake et al., 1979). *V. vulnificus* was isolated from the blood of all 18 cases of primary septicaemia (Tacket et al., 1984). Wound infections are often related to contamination with sea water but are not statistically related to any chronic disease (Blake et al., 1979).

Acute leukaemia and *Candida albicans* infection. Acute leukaemia is often accompanied by iron overload, and patients are unusually susceptible to *C. albicans* infection. Caroline et al. (1969) found a high incidence of raised serum iron in patients with acute leukaemia. Of 34 patients with acute myeloid leukaemia, 21 had high serum iron ($>120\ \mu g\ dl^{-1}$) and 17 had fully saturated transferrin (two of these patients had systemic candidiasis). Another 10 patients had a transferrin saturation of over 50 %. *C. albicans* grew profusely in the serum of patients with acute leukaemia when the serum transferrin was 100 % saturated with iron. The fungus was inhibited by serum with a normal 30 % saturation, but this inhibition was abolished by adding iron to give 100 % saturation. In

addition, it was shown that leukaemic serum plus serum with a high unsaturated iron-binding capacity became inhibitory to the fungus, and that this inhibition could be reversed by adding iron. The same effects were produced using purified human transferrin. Nobody has ever questioned these results, which strongly suggest that the reason for the increased susceptibility of leukaemic patients to *C. albicans* infection is that the fungus is capable of rapid multiplication in tissue fluids containing freely available iron. More recent work has shown that two high-affinity iron permease genes are essential virulence factors in *C. albicans* (Ramanan & Wang, 2000).

Chemotherapy increases iron availability. Chemotherapy can increase levels of freely available iron. One study showed that 70 patients with acute leukaemia had raised levels of transferrin saturation (50–59 %) before treatment. This was increased to 96 % or above after treatment with cytosine arabinoside and daunorubicin together with granulocytopenia (<1000 cells μl^{-1}).There was also a significant fall in total iron-binding capacity. Fungal infections were suspected in 59 % of these patients and confirmed in 19 % (Karp & Metz, 1986). In addition some patients with myeloid leukaemia have circulating low molecular mass iron complexes of about $1 \cdot 0\ \mu mol\ l^{-1}$, which were increased to about $9 \cdot 0\ \mu mol\ l^{-1}$ after chemotherapy (Halliwell et al., 1988).

In another example, six patients with cancers varying from gastric adenocarcinoma to non-Hodgkins lymphoma developed high serum iron within 7 days of starting chemotherapy. Serum iron rose from approximately 77 to 226 µg dl^{-1}, and transferrin saturation from approximately 33 to 97 %. In 12 patients with acute non-lymphocytic leukaemia, the transferrin saturation ranged from 22 to 105 % before treatment. After starting treatment, the saturation rose sharply in 8 out of 9 patients and reached >90 % in all the patients at some time during the observation period of 40 days (Gordeuk et al., 1986). Thus, both leukaemia (Caroline et al., 1969) and chemotherapy (Karp & Metz., 1986) are frequently accompanied by severe iron overload. Since normal human serum is inhibitory for many pathogenic fungi (King et al., 1975; Sutcliffe et al., 1980), including *C. albicans* (Caroline et al., 1969) and *Rhizopus arrhizus* (Boelaert et al., 1993), and since this inhibition is abolished by free iron, it seems highly likely that the removal of this antifungal mechanism is the main reason for the increased susceptibility of patients with leukaemia and those undergoing chemotherapy.

Mucormycosis following treatment with deferoxamine. Boelaert et al. (1993) showed that the iron chelate deferoxamine can act as a siderophore for pathogenic fungi. It promotes mucormycosis in guinea pigs infected with *Rhizopus microsporus*, *R. arrhizus* (formerly called *Rhizopus oryzae*), *Aspergillus fumigatus* and *Cryptococcus neoformans*, but had no significant effect on *C. albicans* infections. It abolished the inhibitory effect of human serum

on *R. microsporus*, and promoted the growth and iron up-take of *R. microsporus* and *R. arrhizus* in human serum. The increased susceptibility of dialysis patients to mucormycosis compared with patients with normal renal function when treated with deferoxamine was explained by the fact that uremia results in sufficient retention of the iron-loaded side-rophore to reverse the fungistatic power of normal serum.

Mucormycosis in diabetic ketoacidosis. Diabetic ketoa-cidosis may be defined as a condition where patients have positive serum ketones and an arterial blood pH of 7·3 or less and/or a serum bicarbonate of 15 mmole l^{-1} or less (Lebovitz 1995; Artis *et al.*, 1982). Artis *et al.* (1982) showed that normal human serum equilibrated with CO_2 to give a pH of 7·4 was inhibitory to *R. oryzae*. Adding extra glucose made no difference, but when the pH was adjusted to 7·3 or below profuse growth of the fungus occurred. In addition, lowering the pH of normal serum from pH 7·4 to 6·35 progressively reduced its iron-binding capacity. When diabetic sera were tested at their clinical pH (6·88–7·21) four out of seven produced profuse growth of the fungus. The remaining three were inhibitory but found to be very low in iron (1–27 µg dl^{-1}). Tests with one of these sera to which iron was added showed that profuse growth occurred at pH 7·08, but no growth at pH 7·4 unless the transferrin was saturated. These results suggest that increased availability of iron during ketoacidosis following a fall in pH is the basic reason for the increased susceptibility of uncontrolled dia-betic patients to mucormycosis. The pH is also critical with other fungi. For example, reducing the pH to 6·6 abolished the inhibitory effect of human serum against *Histoplasma capsulatum* (Sutcliffe *et al.*, 1980).

Thalassaemia and splenectomy. Thalassaemia is char-acterized by severe iron overload and increased suscept-ibility to infection (Hershko *et al.*, 1988). Splenectomy greatly increases the risk in thalassemic patients but this has little effect in normal persons. Thus the rate of infec-tion in 1358 normal persons where splenectomy was per-formed following trauma was 1·5 %, but 7 % in 185 thalassaemia patients (Holdsworth & Cuschieri, 1991). In another 688 patients splenectomised after trauma the inci-dence of infection was 1·5 %, but 25 % in 109 patients with thalassaemia (Singer, 1973).

The incidence of severe infections after splenectomy depends very much on the underlying condition of the patient. There appears to be little risk of infection following splenectomy for trauma, idiopathic thrombocytopenic purpura, extra-hepatic portal vein thrombosis, portal hypertension or localized tumours, but a high risk of fatal infections if splenectomy is accompanied by thalassaemia major, hep-atitis, cirrhosis, Wiskott–Aldrich syndrome, histiocytosis or inborn errors of metabolism (Eraklis *et al.*, 1967).

One of the significant aspects of thalassaemia is that iron overload is greatly increased by splenectomy. Forty-six

patients with HbH (haemoglobin H) disease had a transfer-rin saturation of about 61 % compared with about 36 % in normal controls. In 84 patients with HbE (β-thalassaemia) disease the mean saturation was about 67 %, which rose to 90 % after splenectomy. Among all 130 patients there were 30 with 100 % saturation of their serum transferrin (Pootrakul *et al.*, 1980). Thalassaemic patients also had circulating non-transferrin-bound iron, which was increased by about four-fold after splenectomy (Anuwatanakulchai *et al.*, 1984).

It has been suggested that an increased susceptibility to infection after splenectomy is due to a reduced primary and secondary response to antigens, and a reduced clearance of blood-borne bacteria (Hershko *et al.*, 1988). This is unlikely to be true if splenectomy has little effect following trauma (Holdsworth & Cuschieri, 1991; Singer, 1973), since the same ill effects of splenectomy should apply to trauma patients. Thus, the increase in iron overload following splenectomy of thalassaemic patients has similar con-sequences to that following leukaemia and chemotherapy except that bacterial infections, particularly of *Streptococcus pneumoniae*, predominate in thalassaemia (Holdsworth & Cuschieri, 1991), and fungal infections predominate in leukaemia (Caroline *et al.*, 1969).

Neutropenia and iron overload

In a recent review on fungal infections in neutropenic patients, by Groll & Walsh (2003), it was assumed that neutropenia was the sole cause of the patients' increased susceptibility to fungal infection. No mention is made of the work of Caroline *et al.* (1969) or Boelaert *et al.* (1993) that has shown quite clearly that freely available iron is critically involved in the process. If neutropenia were the sole cause of increased susceptibility one would expect that an increase in the number of polymorphs induced by granulocyte colony stimulating factors would be beneficial. In one case, 4–5 days of treatment gave increased levels of granulocytes, varying from three to eight times base levels, but the authors point out that it was difficult to prove that shorter periods of neutropenia or raised levels of cells were of benefit to the patient (Lieschke & Burgess., 1992). In another instance an increase in neutrophils produced by granulocyte colony stimulating factors failed to demonstrate any significant effect on overall survival or disease-free survival (Dale, 2002). In the review by Groll & Walsh (2003), there was no evid-ence to show that granulocyte colony stimulating factors were of definite clinical value. It may also be significant that patients with pulmonary aspergillosis who had recovered from neutropenia but required further chemotherapy or allogenic cell transplantation had an increased risk of exacerbation of the infection (Groll & Walsh., 2003).

Possible treatment of iron overload

As far as we are aware no adequate clinical trials have yet been made of any method for reducing the iron overload in patients with acute leukaemia, with a view to restoring their normal resistance to infection. This might be done by

treatment with apotransferrin or apolactoferrin, where some observations have suggested that apotransferrin might be useful. Its use prevented the growth of *Staphylococcus epidermidis* in the serum of stem cell transplant patients by binding free iron (Bonsdorf *et al.*, 2003). Tests with two patients showed that apotransferrin could reduce the level of iron saturation from 80–90 % to about 30 % in 12–14 days. Among 20 patients this made no significant difference in the incidence of septic infections compared with controls, but because of these small numbers it is clear that more data are required to assess the true value of reducing iron overload by this means.

Another possibility is to use interleukin-1. Human recombinant interleukin-1 had a powerful effect in rats causing a fall in serum iron from 184 to 24 μg dl^{-1} (87 % fall) in 6 h followed by a slow recovery that was not complete after 24 h. This was accompanied by a well-marked neutrophilia (Uchida *et al.*, 1991). Thus treatment with interleukin-1 would probably provoke many of the other responses associated with fever, but it has the advantage that it is a natural product involved in normal human physiology. It may therefore be safe to use for treatment in spite of its multiple effects.

The importance of haem compounds

Free haemoglobin and derived compounds such as haematin readily provide iron for bacteria (Griffiths & Williams, 1999), and are extremely effective in abolishing natural resistance to infection (Ward & Bullen, 1999). Thus ferric iron, lysed red cells or haematin, were each capable of reducing the lethal intraperitoneal dose of *Escherichia coli* O111 for normal guinea pigs from approximately 1×10^8 to approximately 1×10^4 bacteria, an increase in virulence of approximately 10 000-fold (Bullen *et al.* 1968a). In addition, both lysed red cells and haematin completely abolished the protective effect of specific antibody against 'Pasteurella septica' in mice. In unprotected mice the bacteria grew rapidly to reach viable counts of over 10^9 cells g^{-1} in moribund animals. All the passively immunized animals survived with low bacterial counts that persisted for several days. In passively immunized mice given haematin, protection was abolished and the bacterial counts were identical to those seen in unprotected normal animals. Almost identical results were obtained using lysed mouse cells (Bullen *et al.*, 1968b). It must be emphasized that these overwhelming infections occur in the intact animal where all the cellular phagocytic systems are in place. The cells appear to be overwhelmed by rapid bacterial growth in the extracellular plasma. The same applies to the effect of iron compounds on *E. coli* infections in guinea pigs (Bullen *et al.*, 1968a). Free ferric iron, even in relatively high concentrations (2·2 mM Fe) does not affect the destructive power of polymorphonuclear leukocytes. This occurred only if the cells were exposed to a ferritin–antiferritin complex where iron entered the cell and saturated the iron-binding capacity of the lactoferrin present with subsequent internal growth *Pseudomonas aeruginosa* or *E. coli* (Bullen & Griffiths, 1999).

Blood transfusions. In terms of clinical medicine, it is important to emphasize that normal human plasma contains only 0·3–0·7 mg dl^{-1} of free haemoglobin (Levere *et al.*, 1979). Even this small amount may be caused by damaging some red cells while taking the sample, and the true value may be zero. Any artificial increase in free haemoglobin would be expected to increase susceptibility to infection. Thus blood transfusions, particularly of allogenic cells, may be responsible for this (Ward & Bullen, 1999). In a recent study from an intensive care unit the infection rate among 416 patients transfused with allogenic packed red cells was 15·38 % compared with 2·92 % among 1301 non-transfused patients ($P < 0·005$). There was also a well-marked dose-response pattern. The more red cell units transfused, the greater the chance of infection ($P < 0·0001$) (Taylor *et al.*, 2002). There are many similar articles of this nature, too numerous to discuss in detail, but in general terms it has been reported that transfusion with packed red blood cells increased the risk of ventilator-associated pneumonia in intensive care units (Shorr *et al.*, 2004), and that blood transfusion is an important independent statistical predictor of infection (Agarwal *et al.*, 1993).

This increased susceptibility to infection is often attributed to immune suppression, but there is no certain evidence for this. However, there is no denying that free haem compounds definitely promote infection and the increased availability of iron from the lysis of transfused red cells may be the prime reason for this. This could be augmented by the low molecular mass iron complexes, detectable by the bleomycin assay, that occur in plasma-depleted red blood cell units stored for more than 10 days without haemolysis. (Marwah *et al.*, 2002). In practice, it might be useful to measure the level of free haemoglobin and bleomycin iron in the plasma of transfused patients (Levere *et al.*, 1979; Marwah *et al.*, 2002), to assess the potential risk of circulating free iron.

Blood substitutes. The proposed benefits of blood substitutes based on modified haemoglobin are freedom from infectious agents and the ability to transport oxygen. Unfortunately, modified human haemoglobin is just as effective as native haemoglobin in promoting infection. Intraperitoneal injection of 10^3 *E. coli* O18 K1 into mice resulted in a brief invasion of the blood stream, but the organisms later disappeared and all the animals survived. Animals given 30 mg pyridoxalated glycolaldehyde-polymerized human haemoglobin, immediately before intraperitoneal infection with the same number of organisms, died from septicaemia with bacterial blood counts reaching 10^8 ml^{-1} (Griffiths *et al.*, 1995).

In a clinical trial where patients suffering from severe haemorrhagic shock were given 500–1000 ml 10 % diaspirin cross-linked haemoglobin the death rate after 28 days was 46 % compared with 17 % in those given saline ($P = 0·003$). Although the exact reasons for the increased mortality are not given it is clear that the haemoglobin solution was not a

suitable resuscitation fluid (Sloan *et al.*, 1999). It therefore seems certain that the idea of using free haem compounds as a blood substitute must be abandoned unless some satisfactory way can be found to enclose the material in artificial cells.

Trauma, iron availability and infection

The role of Eh and pH in bacterial growth *in vivo*. It has long been known that normal well-vascularized tissues are highly resistant to infection, and the key to this is adequate oxygenation. Hypoxia is known to favour infection (Hopf *et al.*, 1997). This increased susceptibility to infection is usually attributed to interference with the oxidative killing of bacteria by polymorphonuclear leukocytes (Hopf *et al.*, 1997). This may be true for some bacteria like staphylococci where the percentage of bacteria killed under anaerobic conditions was only 25 % compared with 85 % with air. However, this does not apply to some organisms like *Pseudomonas aeruginosa* or 'Streptococcus viridans', where anaerobic killing was identical to that seen under aerobic conditions, while for others like *Clostridium perfringens* or *Bacteroides fragilis*, the reduction in killing under anaerobic conditions was only slight (5–10 %) (Mandell, 1974). It therefore seems unlikely that the effects of hypoxia can be attributed entirely to a general depression of phagocyte killing against all the different organisms encountered in sepsis. However, hypoxia does have an indirect but profound influence on the availability of iron for bacteria by producing abnormal physical conditions in tissue fluids leading to freely available iron.

In normal well-oxygenated guinea pig muscle the Eh is about $+300$ mV, pH 7·4. Under these conditions the iron bound to transferrin is in the ferric form with an iron saturation of about 30 %. This ensures the extremely low concentration of free ferric iron in tissue fluids such as plasma (about 10^{-18} M), which is essential for natural resistance to infection (Bullen *et al.*, 2005). The Eh and pH of tissue fluids are immensely important since they govern the binding of iron to the protein, which can directly influence bacterial growth. If the Eh falls to -140 mV or below the iron is reduced to the ferrous form and is no longer bound by transferrin. It is then available to the bacteria (Rogers, 1967). The bactericidal effect of human plasma against *E. coli* was abolished by lowering the Eh from approximately $+200$ to -400 mV with a reducing agent. Ferrous iron was detected in the reduced plasma. The bactericidal effect was restored by introducing air into the system and raising the Eh from approximately -400 to $+200$ mV. This shows that exposure to a low Eh does not permanently damage the bactericidal system and that bacterial killing can be switched on or off simply by adjusting the Eh (Bullen *et al.*, 1992). The same applies to the pH of the system whereby the binding of iron by transferrin is progressively lost as the pH and the concentration of bicarbonate ion both fall (Lestas, 1976; Evans *et al.*, 1999). This is accompanied by the loss of bactericidal effects against many different organisms (Bullen & Griffiths, 1999).

The dramatic effect of lowering the pH in abolishing the inhibitory power of serum against *R. oryzae* has already been mentioned. In addition, hypoxia may result in glycolysis and the appearance of lactic acid, which will lead to a fall in tissue pH.

The significance of bacterial reducing systems. An important aspect of infection is the reducing power of bacteria, which is essential for establishing growth *in vivo*. Bacteria bring about reduction as a natural result of metabolic activity. To obtain the energy necessary for proliferation substrates like glucose must be oxidized, and for this purpose free oxygen, combined oxygen, hydrogen acceptors or any suitable type of oxidizing agent will be used by the cell. The intense reducing capabilities of some bacteria are illustrated by *E. coli*, where fifteen million molecules of oxidant may be reduced by one cell in 1 min (Hewitt, 1950).

Hypoxia favours infection and this is dramatically illustrated by the remarkable power of adrenaline to promote bacterial virulence. In skin infections in guinea pigs the injection of 2 µg adrenaline greatly promoted infection with some bacteria. There was a 100 000-fold reduction in the minimum infecting dose of *Clostridium septicum* and *Cl. perfringens*, 10 000-fold with *E. coli*, 60–80-fold with staphylococci, tenfold with *Streptococcus pneumoniae*, but none with *Vibrio cholerae* (Evans *et al.*, 1947). One may ask why the injection of adrenaline should produce such different results with different bacteria. However, adrenaline produces a fall in tissue Eh (-28 to -58 mV) as a result of ischaemia, and the most likely explanation for the varying enhancement of virulence is that bacteria differ greatly in their reducing power. Thus, bacteria like *Cl. perfringens* or *E. coli*, which have very powerful reducing systems capable of reducing the Eh of the medium to -400 mV or below, may be able to take advantage of a reduction in skin Eh to -28 to -58 mV to greatly reduce the number of bacteria necessary for rapid multiplication. Other organisms like *Staphylococcus aureus* have far less powerful reducing systems (Hewitt, 1950) and may be less capable of taking advantage of a reduction in skin Eh. Also, bacteria with powerful reducing systems may be better equipped to lower the Eh of tissue fluids below the critical level of -140 mV where ferrous iron would be freely available and capable of stimulating rapid bacterial growth (Rogers, 1967).

The reducing power of bacteria can have major consequences *in vivo*. Experiments with *Cl. perfringens* in passively immunized animals showed that highly reducing conditions produced by these organisms in peritoneal exudates (approximately -400 mV, pH 6·4 with no free oxygen present) caused a large increase in vascular permeability leading to hypovolaemic shock and death. The exudates were otherwise non-toxic but contained large amounts of protein. Here it was noticeable that relatively high oxygen tensions could be maintained in the dorsal muscles accompanied by a zero oxygen tension and a very low Eh (approximately -400 mV) in the peritoneal fluid (Bullen

et al., 1966). In man, a fall in oxygen tension below 2000–3333 Pa, corresponding to an oxygen saturation of 15–25 % in those with a normal haemoglobin content, is required to produce an increase in vascular permeability in the small vessels of the arm. However, increased permeability will occur if the oxygen tension falls below this value, even for a short period (Henry *et al.*, 1947).

In septic peritonitis in man it has been shown that the mean pH in drainage fluid from 59 patients was 6·75 compared with 7·49 in 105 uninfected patients. The pO_2 in 17 infected patients was zero or near zero (Simmen & Blaser, 1993). Unfortunately, a zero pO_2 does not indicate if reducing conditions exist, and to explore this it would be essential to measure the Eh and pH. Clinically it could be very important to know if highly reducing conditions existed in the peritoneal cavity of these patients.

Gas gangrene. Gas gangrene, following the infection of severe wounds by clostridia, and particularly *Cl. perfringens* type A, is a rapidly spreading myonecrosis and is characterized by a profound toxaemia and prostration (van Heyningen, 1955). During World War II it was generally believed that the alpha toxin of *Cl. perfringens* was primarily responsible for the toxaemia. However, it was found that patients could have a large excess of circulating alpha antitoxin and yet continue to suffer from toxaemia. In nine cases an excess of antitoxin for as long as 92 h before death failed to prevent a fatal outcome (MacLennan & MacFarlane, 1945). It was also a puzzle as to why the bacteria continued to invade antitoxin-containing muscle (Oakley, 1954).

It could be argued that the failure of the antitoxin to protect was because the toxin was rapidly bound to its substrate and access by antitoxin was prevented by mechanical factors. In that case nothing could be done to save the patient, but as van Heyningen (1955) pointed out, the patient could be saved by radical surgery, and the recovery that took place was 'dramatic, heartening, and instructive'. A source of poison had been removed and this did not seem to be the alpha toxin (van Heyningen, 1955).

The reason why the bacteria continued to invade antitoxin-containing muscle may be explained by the very low Eh (Oakley, 1954) in the infected tissue, which would result in the presence of freely available ferrous iron allowing rapid bacterial growth in the presence of normally protective antibody (Bullen *et al.*, 1967). The toxaemia may be explained by the ability of a very low Eh and pH to increase vascular permeability in tissues with the production of fatal shock (Stoner *et al.*, 1967). Both these influences would be removed by radical surgery leading to a rapid recovery, and healthy well-oxygenated tissue containing antitoxin would effectively prevent further spread of the disease. Specific antitoxin is bacteriostatic against *Cl. perfringens* at a relatively high Eh of $+60$ mV, pH 7·4, but this is abolished at an Eh of -140 mV, pH 7·4 or below (Bullen *et al.*, 1964).

Is hyperbaric oxygen the answer?

Adequate oxygenation of tissues is essential for natural resistance to infection (Bullen *et al.*, 2005; Hopf *et al.*, 1997). Clinical experience has shown that increasing the concentration of inspired oxygen to 80 % in patients undergoing colorectal resection, and for 2 h thereafter, halved the incidence of infection compared with patients receiving 30 % oxygen (Grief *et al.*, 2000). With hyperbaric oxygen the level of tissue oxygenation can be increased still further. This has many advantages and has been shown to be effective in many different infections, but it also has side effects that can be troublesome and cannot be ignored (Wang *et al.*, 2003; Gottrup, 2004). Treatment with hyperbaric oxygen would be much more reliable if the Eh and pH of tissues were measured routinely, since this is the only means of telling if reducing conditions exist, or to observe if the Eh can be raised by increasing the oxygen tension. This is one of the most important aspects of infection since restoration of naturally occurring bactericidal systems in infected tissues by controlling the Eh and pH could be of huge benefit.

Conclusions

There is a chain of events in the susceptibility of human tissues to infection that largely or entirely depends upon the availability of freely available iron for the bacteria. Natural resistance to infection operates in an environment where the amount of freely available iron is extremely low. This depends very much on the physical conditions in tissue fluids where the Eh and pH govern the binding of iron to the unsaturated iron-binding proteins transferrin or lactoferrin. The Eh in turn depends on the degree of tissue oxygenation, and therefore hypoxia is of the greatest importance since it produces a fall in tissue Eh, which can result in the production of free ferrous iron and a huge stimulus to bacterial growth. Iron provided by free haem compounds is equally dangerous.

In clinical medicine it might be advantageous to concentrate on those aspects of natural resistance that can be manipulated without the use of antibiotics. This includes a reduction in iron overload during conditions such as acute leukaemia, and in patients undergoing chemotherapy. The use of blood transfusions and blood substitutes based on haemoglobin requires re-examination because these products are potentially dangerous. Finally there is the restoration of natural resistance in injured and infected tissues by increased oxygenation. Measurement and control of the Eh and pH would be essential, but it would be of the utmost value if the bactericidal mechanisms in infected tissues could be restored by purely physical means.

References

Agarwal, N., Murphy, J. G., Cayten, C. G. & Stahl, W. M. (1993). Blood transfusion increases the risk of infection after trauma. *Arch Surg* **128**, 171–176.

Anuwatanakulchai, M., Pootrakul, P. & Thuvasethakul, P. (1984). Non transferrin iron in B-thalassaemia/ Hb E and haemoglobin H diseases. *Scand J Haematol* **32**, 153–158.

Artis, W. M., Fountain, J. A., Delcher, H. K. & Jones, H. E. (1982). A mechanism of susceptibility to mucormycosis in diabetic keto-acidosis: transferrin and iron availability. *Diabetes* **31**, 1109–1114.

Blake, P. A., Merson, M. H., Weaver, R. E., Hollis, D. G. & Heublein, P. C. (1979). Disease caused by a marine vibrio. Clinical characteristics and epidemiology. *N Engl J Med* **300**, 1–5.

Boelaert, J. R., de Locht, M., van Cutsem, J., Kerrels, V., Cantinieaux, B., Verdonck, A., van Landuyt, H. W. & Schneider, Y.-J. (1993). Mucormycosis during deferoxamine therapy is a siderophore mediated infection: in-vitro and in-vivo animal studies. *J Clin Invest* **91**, 1979–1986.

Bonsdorf, L., von Sahlstedt, L., Ebeling, F., Ruutu, T. & Parkkinen, J. (2003). Apotransferrin administration prevents growth of *Staphylococcus epidermidis* in serum of stemcell transplant patients by binding of free iron. *FEMS Immunol Med Microbiol* **37**, 45–51.

Bullen, J. J. & Griffiths, E. (1999). Iron binding proteins and host defence. In *Iron and Infection. Molecular, Physiological and Clinical Aspects*, 2nd edn, pp. 327–368. Edited by J. J. Bullen & E. Griffiths. Chichester: Wiley.

Bullen, J. J., Dobson, A. & Wilson, A. B. (1964). Bacteriostatic effects of specific antiserum on *Clostridium welchii* type A. The role of Eh and pH of the medium. *J Gen Microbiol* **35**, 175–182.

Bullen, J. J., Cushnie, G. H. & Rogers, H. J. (1967). Abolition of the protective effect of *Clostridium welchii* type A antiserum by ferric iron. *Immunology* **12**, 303–312.

Bullen, J. J., Cushnie, G. H. & Stoner, H. B. (1966). Oxygen uptake by *Clostridium welchii* type A, its possible role in experimental infections in passively immunised animals. *Br J Exp Pathol* **47**, 488–506.

Bullen, J. J., Leigh, L. C. & Rogers, H. J. (1968a). The effect of iron compounds on the virulence of *Escherichia coli* for guinea pigs. *Immunology* **15**, 581–588.

Bullen, J. J., Wilson, A. B., Cushnie, G. H. & Rogers, H. J. (1968b). The abolition of the protective effect of *Pasteurella septica* antiserum by iron compounds. *Immunology* **14**, 889–898.

Bullen, J. J., Rogers, H. J. & Griffiths, E. (1978). Role of iron in bacterial infection. *Curr Top Microbiol Immunol* **80**, 1–35.

Bullen, J. J., Spalding, P. B., Ward, C. G. & Gutteridge, J. M. C. (1991). Haemochromatosis, iron, and septicaemia caused by *Vibrio vulnificus*. *Arch Intern Med* **151**, 1606–1609.

Bullen, J. J., Spalding, P. B., Ward, C. G. & Rogers, H. J. (1992). The role of Eh, pH, and iron in the bactericidal power of human plasma. *FEMS Microbiol Lett* **94**, 47–52.

Bullen, J. J., Rogers, H. J., Spalding, P. B. & Ward, C. G. (2005). Iron and infection, the heart of the matter. *FEMS Immunol Med Microbiol* **43**, 325–330.

Caroline, L., Rosner, F. & Kozinn, P. J. (1969). Elevated serum iron, low unbound transferrin and candidiasis in acute leukaemia. *Blood* **34**, 441–451.

Chart, H. & Griffiths, E. (1985). The availability of iron and the growth of *Vibrio vulnificus* in sera from patients with haemo-chromatosis. *FEMS Microbiol Lett* **26**, 227–231.

Dale, D. C. (2002). Colony-stimulating factors for the management of neutropenia in cancer patients. *Drugs* **62**, 1–15.

Eraklis, A. J., Brammer, S. R., Diamond, L. K. & Gross, R. E. (1967). Hazard of overwhelming infection after splenectomy in childhood. *N Engl J Med* **267**, 1225–1229.

Evans, D. G., Miles, A. A. & Niven, J. S. F. (1947). The enhancement of bacterial infections by adrenaline. *Br J Exp Pathol* **29**, 20–39.

Evans, R. W., Crawley, J. B., Joannou, C. L. & Sharma, N. D. (1999). Iron proteins. In *Iron and Infection. Molecular, Physiological, and Clinical Aspects*, 2nd edn, pp. 27–86. Edited by J. J. Bullen & E. Griffiths. Chichester: Wiley.

Gordeuk, V. R., Brittenham, G. M., McLaren, G. D. & Spagnuolo, P. J. (1986). Hyperferraemia in immunosuppressed patients with acute nonlymphatic leukemia and the risk of infection. *J Lab Clin Med* **108**, 466–472.

Gottrup, F. (2004). Oxygen in wound healing and infection. *World J Surg* **28**, 312–315.

Grief, R., Akca, O., Horn, E.-P. & Sessler, D. I. (2000). Supplemental perioperative oxygen to reduce the incidence of surgical wound infection. *N Engl J Med* **342**, 161–167.

Griffiths, E. & Williams, P. (1999). The iron-uptake systems of pathogenic bacteria, fungi and protozoa. In *Iron and Infection. Molecular, Physiological, and Clinical Aspects*, 2nd edn, pp. 87–212. Edited by J. J. Bullen & E. Griffiths. Chichester: Wiley.

Griffiths, E., Cortes, A., Gilbert, N., Stevenson, P., MacDonald, S. & Pepper, D. (1995). Haemoglobin-based blood substitutes and sepsis. *Lancet* **345**, 158–160.

Groll, A. H. & Walsh, T. J. (2003). Invasive fungal infections in the neutropenic cancer patient. *Abstr Hematol Oncol* **6**, 18–26.

Gutteridge, J. M. C., Rowley, D. A., Griffiths, E. & Halliwell, B. (1985). Low-molecular-weight complexes and oxygen radical reactions in idiopathic haemochromatosis. *Clin Sci* **68**, 463–467.

Halliwell, B., Aruoma, O. I., Mufti, G. & Bomford, A. (1988). Bleomycin detectable iron in serum from leukaemic patients before and after chemotherapy. *FEBS Lett* **241**, 202–204.

Henry, J., Goodman, J., Meehan, J. & Frankel, R. (1947). Capillary permeability in relation to acute anoxia and to venous oxygen saturation. *J Clin Invest* **26**, 1119–1129.

Hershko, C., Peto, T. E. A. & Weatherall, D. J. (1988). Iron and infection. *BMJ* **296**, 660–664.

Hewitt, L. F. (1950). *Oxidation–reduction Potentials in Bacteriology and Biochemistry*, 6th edn, pp. 97–127. Edinburgh: E&S Livingstone.

Holdsworth, R. J., Irving, A. D. & Cuschieri, A. (1991). Post-splenectomy sepsis and its mortality rate: actual versus perceived risks. *Br J Surg* **78**, 1031–1038.

Hopf, H. W., Hunt, T. K., West, J. M., & 9 other authors (1997). Wound tissue oxygen tension predicts the risk of wound infection in surgical patients. *Arch Surg* **132**, 977–1004.

Karp, J. E. & Metz, W. G. (1986). Association of reduced total iron binding capacity and fungal infections in leukemic granulocytopenic patients. *J Clin Oncol* **4**, 216–220.

King, R. D., Kahn, H. A., Foye, J. C., Greenberg, J. H. & Jones, H. E. (1975). Transferrin, iron and dermatophytes. 1. Serum dermatophyte inhibitory component definitively identified as unsaturated trans-ferrin. *J Lab Clin Med* **86**, 204–212.

Lebovitz, H. E. (1995). Diabetic ketoacidosis. *Lancet* **345**, 767–772.

Lestas, A. N. (1976). The effect of pH on human transferrin, selective labelling of the two iron-binding sites. *Br J Haematol* **32**, 341–350.

Levere, R. D., Swerdlow, F. & Garaway, M. R. (1979). Measurement of human plasma haemoglobin by difference spectrophotometry. *J Lab Clin Med* **77**, 168–176.

Lieschke, G. J. & Burgess, A. W. (1992). Granulocyte colony stimulating factor and granulocyte-macrophage colony-stimulating factor. *N Engl J Med* **327**, 28–35.

MacLennan, J. D. & MacFarlane, R. W. (1945). Toxin and antitoxin studies of gas gangrene in man. *Lancet* **2**, 62–66.

Mandell, G. I. (1974). Bactericidal activity of aerobic and anaerobic polymorphonuclear neutrophils. *Infect Immun* **9**, 337–341.

Marwah, S. S., Blann, A., Harrison, P., Lumley, M. A., Wright, J., McDowell, J., Phillips, J. D., Rea, C. & Bareford, D. (2002). Increased non-transferrin bound iron in plasma-depleted SAG-M red blood cell units. *Vox Sang* **82**, 122–126.

McLaren, G. D., Muir, W. A. & Kellermeyer, R. W. (1983). Iron overload disorders, natural history, pathogenesis, and therapy. *Crit Rev Clin Lab Sci* **19**, 205–266.

Murray, B. E. (2000). Vancomycin resistant enterococcal infections. *N Engl J Med* **342**, 710–721.

National Institute of Allergy & Infectious Diseases (2004). *Fact Sheet: The Problem of Antibiotic Resistance.* http://www.niaid.nih.gov/factsheets/antimicro.htm

Oakley, C. L. (1954). Gas gangrene. *Br Med Bull* **10**, 52–58.

Pootrakul, P., Rugkiatsakul, R. & Wasi, P. (1980). Increased transferrin saturation in splenectomised thalassemic patients. *Br J Haematol* **46**, 143–145.

Ramanan, N. & Wang, Y. (2000). A high affinity iron permease essential for *Candida albicans* virulence. *Science* **288**, 1062–1064.

Rogers, H. J. (1967). Bacteriostatic effects of horse sera and serum fractions on *Clostridium welchii* type A, and the abolition of bacteriostasis by iron salts. *Immunology* **12**, 285–301.

Shorr, A. F., Duh, M.-S., Kelly, K. M. & Kolief, M. H. (2004). Red blood cell transfusion and ventilator-associated pneumonia, a potential link? *Crit Care Med* **32**, 666–674.

Simmen, H.-P. & Blaser, J. (1993). Analysis of pH and pO_2 in abscesses, peritoneal fluid, and drainage fluid in the presence or absence of bacterial infection during and after abdominal surgery. *Am J Surg* **163**, 24–27.

Singer, D. B. (1973). Postsplenectomy sepsis. *Perspect Pediatr Pathol* **1**, 285–311.

Sloan, E. P., Koenigsberg, M., Gens, D., Cipolle, M., Runge, J., Mallory, M. N. & Rodman, G., Jr (1999). Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe haemorragic shock: a randomised controlled efficacy trial. *JAMA* **282**, 1857–1864.

Stoner, H. B., Bullen, J. J., Cushnie, G. H. & Batty, I. (1967). Fatal intraperitoneal infection with *Clostridium welchii* type A in passively immunised guinea-pigs. The effect on vascular permeability. *Br J Exp Pathol* **48**, 309–318.

Sutcliffe, M. C., Savage, A. M. & Alford, R. H. (1980). Transferrin-dependent growth inhibition of yeast-phase *Histoplasma capsulatum* by human serum and lymph. *J Infect Dis* **142**, 209–219.

Tacket, C. O., Brenner, F. & Blake, P. A. (1984). Clinical features and an epidemiological study of *Vibrio vulnificus* infections. *J Infect Dis* **149**, 558–561.

Taylor, R. W., Manganaro, L., O'Brien, J., Trottier, S. J., Parkar, N. & Veremakis, C. (2002). Impact of allogenic packed red blood cell transfusions on nosocomial infection rates in the critically ill patient. *Crit Care Med* **30**, 2249–2254.

Uchida, T., Yamagiwa, A. & Nakamura, K. (1991). The effect of interleukin-1 on iron metabolism in rats. *Eur J Haematol* **46**, 1–5.

van Heyningen, W. E. (1955). The role of toxins in pathology. In *Mechanisms of Microbial Pathogenicity* (Society for General Microbiology Symposium no. 5), pp. 17–39. Edited by J. W. Howie & A. J. O'Hea. Cambridge: Cambridge University Press.

Vincent, J.-L., Chierego, M., Struelens, M. & Byl, B. (2004). Infection control in the intensive care unit. *Expert Rev Anti Infect Ther* **2**, 795–805.

Wang, C., Schwaitzberg, S., Berliner, E., Zarin, D. A. & Lau, J. (2003). Hyperbaric oxygen for treating wounds, a systematic review of the literature. *Arch Surg* **138**, 272–279.

Ward, C. G. & Bullen, J. J. (1999). Clinical and physiological aspects. In *Iron and Infection. Molecular, Physiological and Clinical Aspects*, 2nd edn, pp. 369–450. Edited by J. J. Bullen & E. Griffiths. Chichester: Wiley.