Artigo de Opinião / Opinion Article

IRON AND THE PARADOX OF DISEASE *

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I shall start by travelling first to the turning of the last century. In Art, at that time, an atmosphere of multidisciplinarity, brought sculptors, architects, cabinet makers, silversmiths, glass blowers together to create buildings, spoons, knives, forks, chairs, lamps, that would bring a harmonious beauty to daily life. This movement reflected a reaction to the monotony brought about by the mass production of the industrial revolution. In dictionaries of the time, however, the word multidisciplinarity cannot be found, perhaps because artists did not consider their craft a discipline.

Science had a role in this movement, not as a discipline either, but by "opening new horizons from all sides", to cite Émile Gallé (1), revealing to the artist the beauty of forms in plants, flowers, insects, deep sea living forms, etc. Artists incorporated in their work the representation of those forms (Figure 1).

The discovery of the X-rays by Roentgen and the discovery of radioactivity by the Curies, must have brought also to medical science an atmosphere of magic and revelation. The revelation of forms hitherto unseen but easily recognised by all (Figure 2). The medical sciences must also have brought much hope and promise in that period. After years of meticulous mapping the distribution of infectious diseases in the world and in cities, in an attempt to understand and control their propagation

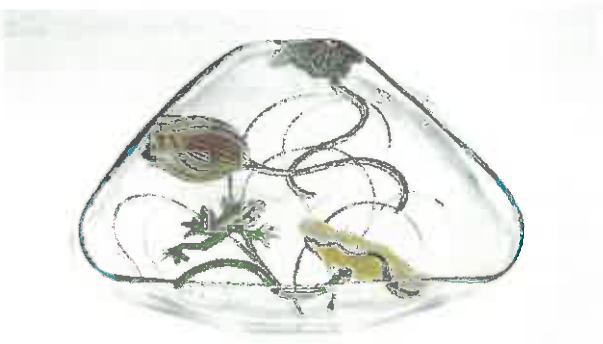

Figure 1 - Cup, Emile Gallé, Musée d'Orsay (reproduced from card).

(2), successful vaccines and the consequent concept of immunity must have transmitted a collective sense that individual prevention of lethal diseases was at hand.

Far away from the practical benefit of vaccines to survival and the contribution of X-rays to diagnosis, the contributions of two other men very different from Pasteur and Roentgen, were to influence us to this day. Indeed the influences of Charles Darwin and of Claude Bernard reach this hour and what we have to say in this session. Darwin took our ancestry away from God and indirectly strengthened St Francis of Assisi's message of the brotherhood of man with other species. We see how in 2000, genes identified in the worm promise understanding of some of the most frequent diseases among the ageing human population (3). Claude Bernard placed man firmly at the boundary between two environments: the external and the internal milieu. Bernard did something else, he viewed the importance of the maintenance of the stability of the internal milieu as a fundamental condition for the autonomy of organisms facing the adversity and the vicissitudes of the external environment (4). A notion illustrated in Figure 3.

This brings me to the title of this talk: iron and the paradox of disease. Introducing the importance of iron is

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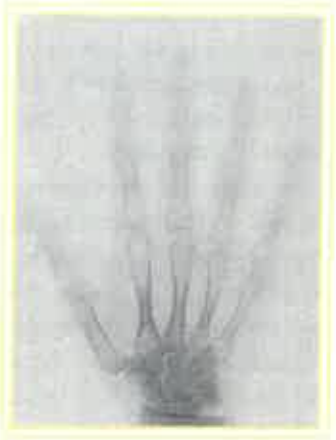


Figure 2 - X-ray of hand, with permission of Rosa Lacerda, the owner.

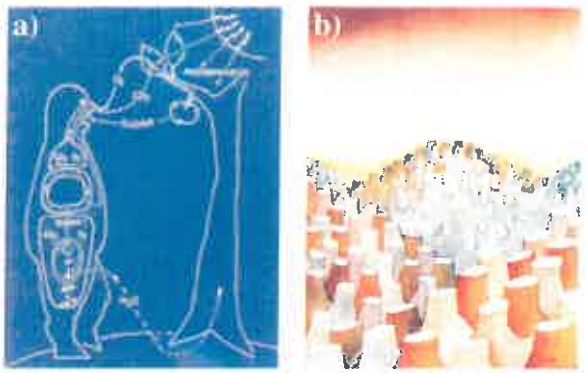


Figure 3 - Illustration of the dependence of man from the external environment (a). Importance of the autonomy of the internal milieu to survive in extremely adverse conditions (b). (a) Modified from ref. 5. (b) Reproduced from ref. 6, with permission.

perhaps not difficult to those outside science or to those outside the field. Everybody seems to have been exposed to the notion that iron deficiency is something to avoid. The manifestations of iron deficiency are in every day life, fatigue, shortness of breath, a feeling of weakness and in a laboratory analysis, low haemoglobin levels, low numbers of red blood cells, in summary, anaemia.

The importance of iron in evolution must have been such that higher vertebrates have developed an almost perfect system of recycling and storing an element difficult to find in the external environment (reviewed in ref. 7).

The total body iron in a 70kg man has been estimated to be 4.2g distributed as follows: haemoglobin (74.3%), ferritin (16.4%), myoglobin (3.3%), haptoglobin-haemoglobin (0.2%), catalase (0.11%), cytochrome *c* (0.08%), transferrin (0.07%). Thus, the great majority of iron in the body is within the red blood cells followed, by the liver where iron is stored.

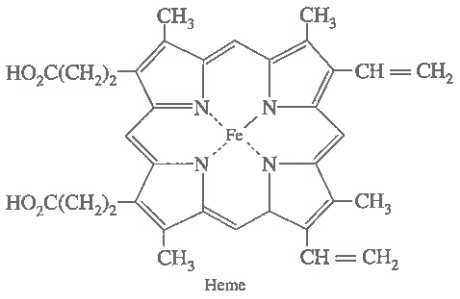
The iron in haemoglobin in the circulating red blood cell (Figure 4) is the key to the exchange of oxygen between the internal and the external environments and to the distribution of oxygen to all tissues. The exact molecular basis of oxygen transport and delivery is still not quite fully understood but it is clear that iron is a critical component both to the effector process and to the regulation of the synthesis of haemoglobin.

Iron sits at the centre of the porphyrin ring, a structure identical to the one found in chlorophyll, except for the metal at the centre (Figure 5).

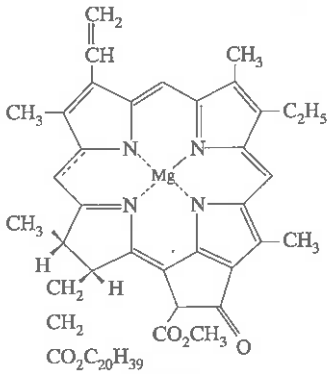
Iron, however, is not easily available in the external environment. In evolutionary terms and combining Darwin and Bernard's messages, Nature selected a system of recycling iron that makes higher vertebrates autonomous and independent of the amounts available in the external environment. The system is highly regulated and when stores are low absorption increases. Recycling of the iron in haemoglobin occurs mostly in the spleen through the selective removal of senescent red blood cells by the macrophages. After phagocytosis of the red blood cells, the iron reenters the circulation pool which takes it to the bone marrow for erythropoiesis (see also Figure 4). Indeed it has been estimated that for all practical purposes iron deficiency anaemia is about impossible. *In a normal adult male, for iron deficiency to occur iron loss must be increased.* Assuming that iron is not absorbed and 1mg excreted per day, it would take 6 years for negative balance to take place. This theoretical estimation stands in manifest contrast with WHO numbers identifying 500-600 million people with iron deficiency anaemia (7).

From a linear evolutionary view it is therefore paradoxical that there is iron deficiency in our species. From a

Figure 4 - Main steps of the iron recycling and storage. Modified from ref. 8.



Heme



Chlorophyll

Figure 5 - The porphyrin ring is an example of the constancy of a molecule in evolution. Except for the difference in the metal at the centre, Fe and Mg, both haemoglobin and chlorophyll contain a porphyrin ring as a key structure for their function.

complex social interactive model it is not: 1) In certain parts of the world periods of famine, or hunger, or malnutrition are lasting longer than 6 years; 2) Women are loosing blood through multiple pregnancies in those same and in other parts of the world; 3) Bacteria and parasites make use of the stability of iron levels and haemoglobin levels in one species for their own survival; 4) Finally, one of the first responses of the immune system to infection is the production of cytokines that have as one effect to lower iron levels and diminish the making of red blood cells (erythropoiesis) (9).

It is equally paradoxical that iron overload can constitute a problem. As mentioned earlier, the system is highly regulated and when stores are replete, absorption stops. If intake continues because iron rich diets are for example given to animals in captivity, dietary iron overload can develop with lethal consequences in some species (7). Dietary iron overload has also been recorded in man. The form of iron overload that we have been studying in Oporto and that brings us to this Symposium, is the genetic disease, i.e., Genetic Haemochromatosis. In this disease, the fine mechanism of regulation of iron entry fails and iron keeps being absorbed in spite of replete stores. The consequent changes are a higher mean volume of the red blood cell and, with the slow progression of entry, the undesirable accumulation of iron in target organs, such as the liver, the pancreas, the heart and the joints (10, Figure 6).

The gene for this disease in the Caucasian population was discovered in 1996 (11). What brought us to study it, however, was a hypothesis, published in 1978, 100 years after Claude Bernard's statement about the stability of the internal milieu (12,4). The hypothesis postulated that the immunological system, in addition to its well-established protection from the invasion of pathogens from the external environment, had a function of protecting the internal milieu from the accumulation of iron in tissues. The accumulation of iron in cells, paradoxically, is toxic. Iron overload, unlike iron deficiency, in addition to symptoms of fatigue, has clinical expression related to the organs where it accumulates: the liver, the pancreas, the joints, the heart.

To be true, such a hypothesis expected to find abnormalities of the immunological system, in the patients with genetic haemochromatosis. The contribution of my research group consists in the finding of such abnormalities in the human disease and the subsequent confirmation of the existence of hemochromatosis in mice deficient in certain lymphocyte populations (13,14). A contribution to the clinical study of this disease and to the more general principle that the immunological system can have a regulatory role in physiology, in addition to its role in protection from infection. The way the normal

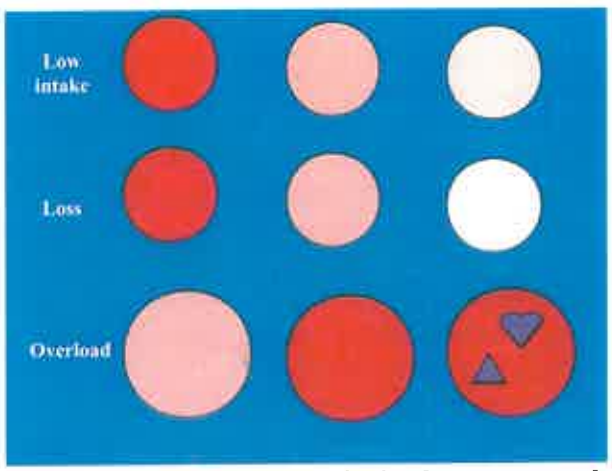


Figure 6 - Representation of slow or fast iron loss processes resulting from low intake, loss from haemorrhage or redirection to parasites. Failure of regulation of iron absorption in genetic haemochromatosis results in higher mean corpuscular volume (MCV, represented by the larger diameter of the red circles), and accumulation in the liver (triangle) and heart (blue).

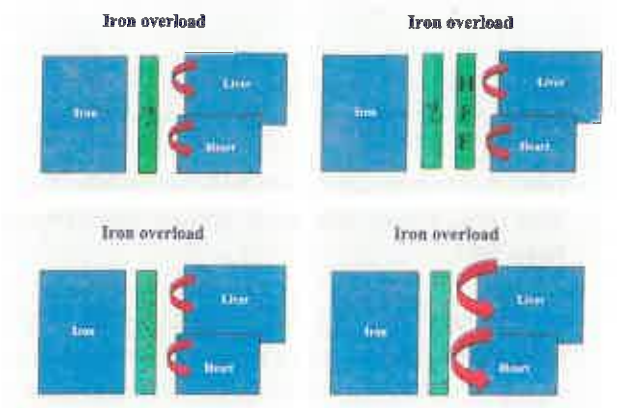


Figure 7 - Discovery of the gene for haemochromatosis (HFE) did not resolve the question of individual variation in the clinical expression of the disease (?). Our work has demonstrated a consistent relationship between a lower number of lymphocytes (yellow circles) and a more severe iron accumulation in tissues (thicker red arrows). Diagram conceived by E Cruz.

or the defective numbers of lymphocytes relate to the expression of iron overload is shown schematically in Figure 7.

The evidence that a gene defect was insufficient to explain the variation in the expression of the disease was available much before the gene was discovered from the careful analysis of the quantities of iron accumulated with age. A genetic disease that results in the slow accumulation from birth of iron in tissues is expected to relate to age. As illustrated in Figure 8 this is not the case. Younger patients can have higher indices of iron accumulation than much older patients (15).

In a study that has now lasted 15 years and examined close to 400 people, including patients and family members, we have found that patients with low numbers of a certain type of lymphocytes have higher iron accumulation (Figure 7). Moreover, Manuela Santos, an independent PhD student in Hans Clevers lab, demonstrated that a severe form of iron overload seen in mice defective in lymphocytes could be treated by replacing the missing cells with cell transfers (14). More recently, Levy et al., studying the iron overload of mice without the gene first discovered in humans crossed with other mice deficient in a selected lymphocyte population, observed a more severe overload in the latter, called double knockout mice (16, Figure 9). Their interpretation of the results involves the action of modifier genes rather than the immunological system as a modifier system. Time and more experiments will tell whether those putative genes are genes that regulate lymphocyte numbers.

I am thus closing this contribution to the Expo2000 with

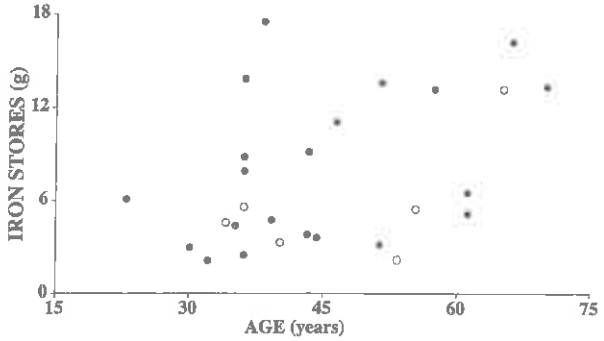


Figure 8 - Evidence that factors other than progressive accumulation from disregulation of iron absorption must contribute to tissue iron overload. Progressive accumulation should result in increase with age. That is not the case. Data from Porto et al., (15).

the presentation of a novel view of the function of the immunological system. Preliminary work in collaboration with Sá Miranda studying the same lymphocyte populations in patients with Gaucher disease showed that severity of bone lesions in those patients could also be related to T lymphocyte numbers (17). Saraiva's group has recently found evidence of activation of inflammation genes in patients with peripheral neuropathies due to the accumulation of amyloid (18).

The problem of the individual variation in the phenotypic expression of disease is common to all clinical researchers working on genetic diseases. It raises fundamental questions about modifier "screens". It is improbable that these screens are just one or two genes. The immunological system as a system of circulating cells or genes of the immunological system, may be just one answer. To have a gene mutation that can be, is, or has

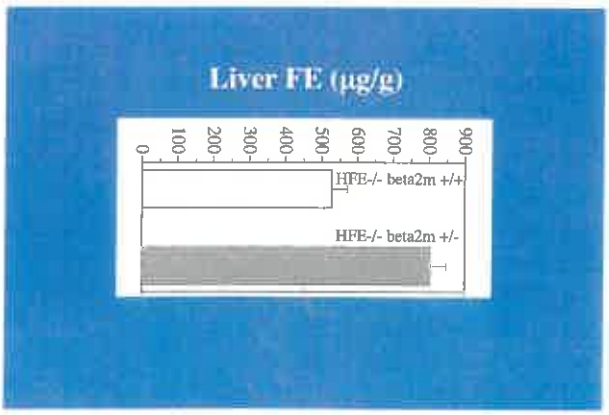


Figure 9 - Double knock-out technology permits to demonstrate that the absence of some cell populations or genes, has a modifier effect of degree of iron overload measured in the liver of HFE knockout mice, crossed with mice with (ß2m+/+) or without (ß2m-/-) certain types of lymphocytes (modified from ref. 16).

been associated with a particular disease, means that the person carrying it can take a greater care in the surveillance of his or of her health. *It does not mean that he or she will necessarily come to see the clinical expression of that disease*. In Portugal, for example, every person carrying a gene mutation is more likely to die in a car accident than from his/her genetic abnormality. Helas.

Bernard considered the nervous system as a possible key system in the regulation of the stability of the internal milieu. He could not have foreseen a role for the immunological system. In the late XIX century the notion of immunity was very real but there was no immunological system. The immunological system as we are still unravelling it today, has its roots in the 1960s.

I have wondered whether because the concept of immunity was well established by the beginning of the XX century, we have lived 100 years thinking that the immunological system is exclusively for the purpose of immunity.

Nothing in the last twenty years has made us cease to consider that the immunological system has a role in the physiology of other systems, in particular the metabolism of iron. The fact that the haemochromatosis gene belongs to one of the most important loci of the genetics of the immunological system (11) makes the two systems inseparable also at the genetic level.

Graça Porto will talk of the gene (19). Jost Schönberger will tell us about genes that can break the heart (20). We invited him in the 1900 artistic sense of enlarging our horizons with the experience of others. The target organ of a genetic disease, in his case, is the heart. Indeed one other serious aspect of our ignorance resides in not knowing what determines the selectivity of target organs for the expression of genetic disease.

In summary, we seem to know a lot more than our colleagues knew at the turning of the last century. But what we know for sure better than they did, is how little we know (21).

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21. Oppenheimer PL. Comments on Science in the 1900. Letter, 2000. *"Dear Maria, If you are going to talk of science in 1900 (ie. physics) be careful, as every one will be repeating the same story of how in 1900 the general view was that everything in physics had been discovered and that all that remained was to calculate things out to a few more decimal places. Quantum Mechanics put an end to this (Relativity also). Even today some physicists believe something like this - for ex. Hawking is his Inaugural lecture of 1980 says that it is quite possible that by the end of the century physicists will have a complete theory that will describe everything, and thus there will be nothing fundamental left to find out in physics. God knows what he would say now. Anyway, this comparison, will be used and abused by everyone, so you may try to find others that are as important and not as well known. Love, Peter"*