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Effects of prolonged iron loading in the rat using both parenteral and dietary routes.

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Female Porton rats have been treated with either parenteral iron (intraperitoneal red cells) or dietary iron (carbonyl iron) for up to 12 months or 22 months respectively. In the parenteral iron loaded animals, the liver iron concentration rose from approximately 2 mg g^{-1} dry wt at 2 months to 21 mg g^{-1} dry wt at 12 months, while for the dietary iron loaded animals, this value rose from 14 to 48 mg g^{-1} dry wt at 12 months to over 60 mg g^{-1} dry wt after 22 months. In contrast, splenic iron concentrations rose more in the parenterally loaded animals (up to 66 mg g^{-1} dry wt after 12 months) than in the dietary loaded animals (approx. 34 mg g^{-1} dry wt after 24 months). This study yielded hepatic iron concentrations comparable to those seen in human thalassaemia patients with comparative low hepatotoxicity. Splenic iron concentrations in the parenteral iron loaded group generally exceeded those reported in thalassaemia. Iron concentrations derived from computer assisted morphometry of liver iron deposits correlated well ($r = 0.88$, $p < 0.001$) with chemical analysis data. The fraction of iron in the non-parenchymal cells correlated positively with the duration of iron loading ($r = 0.86$, $p < 0.001$).

Keywords: animal model, haemochromatosis, iron overload, iron toxicity, morphometry, thalassaemia

Introduction

Normally, the amount of iron in the human body is closely regulated, with a balance being achieved between the amount lost and that absorbed from the diet (Jacobs 1977). However, certain disease states disturb this balance and can lead, either directly or indirectly, to the accumulation of large amounts of iron in the body. Chief among these diseases are thalassaemia and haemochromatosis. The former is a generic term used to describe a series of genetic abnormalities resulting in the ineffective synthesis of haemoglobin, which if untreated, can lead to chronic anaemia, in turn causing the increased absorption of iron from the diet (Halliday & Powell 1982). The latter is also a genetic problem, but directly relating to the control of the absorption of iron rather than haemoglobin synthesis (Feder *et al.* 1996; Powell & Jazwinska 1996).

The prevalence of these genetic diseases, which particularly in the case of thalassaemia is the cause of very serious public health problems in certain parts of the world (Fucharoen & Winichagoon 1992), has lead to the development of a series of models of iron overload (Ramm 1998). These have mainly involved the use of iron chelates such as iron dextran (see e.g. Golberg, Smith & Martin 1957, Adam & Schwartz 1993), ferric nitrilotriacetate (Awai *et al.* 1979; Preece *et al.* 1989) and iron sorbitol (Hultcrantz & Arborgh 1978) delivered parenterally,

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while dietary iron loading has focused on the use of carbonyl iron (see e.g. Bacon *et al.* 1983; Park *et al.* 1987), and more recently ferrocence (Longueville & Crichton 1986; Ward *et al.* 1991). Unfortunately, to date many of these studies have been relatively short term, that is, only loading for a matter of weeks, and generally have not produced the same degree of iron loading as found in humans in the latter stages of the two disease states.

The aim of the present study was to produce animal models in which iron loading could be achieved to the same degree as in non-treated human patients. In addition, because of the nature of the two disease states, human sufferers become iron-loaded either through the diet (haemochromatosis and untreated thalassaemics) or through repeated blood transfusions (treated thalassaemics), both parenteral and dietary iron loading were trialed. The results from parenteral iron loading, achieved through the injection of intact red blood cells (Morgan 1961), have been compared with data obtained from dietary iron loading, achieved using 2.5% carbonyl iron (Bacon *et al.* 1983). These two model systems were established over a prolonged time period so that similar levels of iron loading to those found in human disease states (without chelation) could be obtained in order to determine whether the route of iron entry into the body affects both the location in which the iron is deposited and the ultimate concentration. Computer assisted morphometic analysis of histological sections was also used to measure iron in different cell populations indirectly. It has been suggested previously that this technique may well prove invaluable in a clinical setting in the future for the quantitative measurement of stainable hepatic iron (Deugnier *et al.*, 1982).

Material and methods

Animals and iron loading

Female Porton rats (obtained from the Flinders Medical Centre Animal House, Bedford Park, SA, Australia), were used for all experimental procedures. Following previously published procedures (Olynyk *et al.* 1992), these animals were the offspring of iron loaded mothers who, in turn, had been iron loaded during pregnancy and lactation with carbonyl iron (AR, Sigma Chemical Company, St. Louis, Miss.) Rats were housed in polyethylene cages with stainless steel wire tops and were allowed food and water *ad libitum*. All animal experimentation was conducted under the auspices of the Murdoch University Animal Ethics Committee.

The experimental animals were further divided into 3 groups, namely: control, parenteral-iron loaded, and dietary-iron loaded. Rats in the control group received a normal regular commercial diet after weaning and were sacrificed at 2, 4, 6, 8 and 12 months of age.

Parenteral iron loading was achieved through the use of regular intraperitoneal blood injections. Iron loading, by weekly injections of washed, packed red blood cells (Morgan 1961), was started when the rats were 1 month old. For the first 3 months, 2 ml of packed red blood cells 100 g^{-1} body weight were injected per week (Hershko *et al.* 1978). After 3 months, the injection volume was increased to 4 ml of packed red cells 100 g^{-1} body weight but was administered fortnightly. The rats were sacrificed at intervals of 2, 4, 6, 8, 10 and 12 months. In addition, injections to two rats were stopped at 10 months for 1 and 2 months and these animals were sacrificed 1 month and 2 months later, respectively.

Packed red blood cells used for the injections were obtained from male and female donor rats obtained from the same colony as the experimental animals. Following euthanasia in 80% CO_2 : 20% O_2, blood was taken from the donor by heart puncture and kept for a maximum of 28 hours in sterile heparinised vacuum tubes (Vacutainer®, Becton Dickinson, England). The heparinised blood was centrifuged (100 g 20 min 10°C) to remove the plasma and buffy coat, then washed and resuspended three times with 0.9% NaCl solution prior to injection.

The dietary iron group received an iron supplement of 2.5% carbonyl iron (w/w). This was achieved by moistening normal rat pellets with water and then sprinkling over 2.5% carbonyl iron (w/w) and mixing well to provide an even covering on the pellets. These were then allowed to dry before being substituted for the normal feed. The animals readily accepted the iron-coated pellets. Animals from this group were sacrificed at regular intervals up to 22 months. In several cases towards the end of the experimental period, individual rats started to show signs of iron toxicity. It was deemed appropriate to sacrifice these animals immediately, thus producing slightly irregular sample dates. At 22 months, four rats were returned to a regular diet for a further 2, 4, 6 and 8 weeks and were therefore sacrificed at 22.5, 23, 23.5 and 24 months old, respectively.

Haematological, histological and iron determinations

Blood samples were obtained by cardiac puncture. Haemoglobin concentration, haematocrit, and red blood cell indices were measured using an automatic cell counter (Coulter Counter®, Coulter Electronics Ltd, Harpenden, England). A reticulocyte count for each blood sample was obtained manually by counting the number of reticulocytes in 1000 red blood cells, following incubation with methylene blue for 30 mins.

After an abdominal incision, the liver and spleen were removed, weighed and then rinsed with 0.9% NaCl to remove excess blood. A small sample of each organ was fixed immediately in 10% neutral formalin in phosphate buffer (0.2 M, pH 7.4) for routine histological processing. Sections (4 μm) were cut and stained either with haematoxylin and eosin for histopathological evaluation, or using

potassium ferrocyanide (Perls' technique) for iron(III) (Bancroft & Cook 1984). The amount of stainable iron in the hepatocytes was graded subjectively by light microscopy using the method of Scheuer *et al.* (1962). In this grading system, '0' refers to no stainable iron in hepatocytes, while '4' refers to the highest level of iron loading with 100% of the hepatocytes containing iron. A similar system was established to grade the degree of iron loading in the Kupffer cells (non-parenchymal cells) in the liver and in the macrophages in the spleen. In these cells, iron loading was graded from no stainable iron through trace, mild, moderate to marked. An additional portion of both liver and spleen was removed and immediately fixed overnight with 2.5% gluteraldehyde in phosphate buffer (0.2M, pH 7.40, 4°C) followed by postfixation in 2% osmium tetroxide in phosphate buffer, then dehydrated and embedded in epon/araldite. Ultrathin sections were cut using a Reichart ultramicrotome, prior to examination using a Philips 301 electron microscope.

The remaining portions of liver and spleen from each rat were quickly frozen and then freeze dried. Each freeze-dried sample was then ground to a powder to ensure thorough mixing and sub-samples taken for atomic absorption spectroscopy. Freeze-dried sub-samples, together with standard reference material (bovine liver standard reference material 15779), were then digested using nitric/perchloric acid and the iron content determined using a Varian AA-175 series spectrophotometer.

Computerised morphometry

The amount of hepatic iron in Perls' stained sections was measured using a Video Pro 32 Colour Image Analysis System (Leading Edge Pty. Ltd, Science Park Adelaide, Bedford Park SA 5042, Australia) (Jarvis, 1988). Prior to any measurements being taken, the background light intensity was established for each slide in order to standardise any effect that minor variations in the slide, cover slip or section thickness might have had. The total area of the tissue stained blue (i.e. containing iron) and the

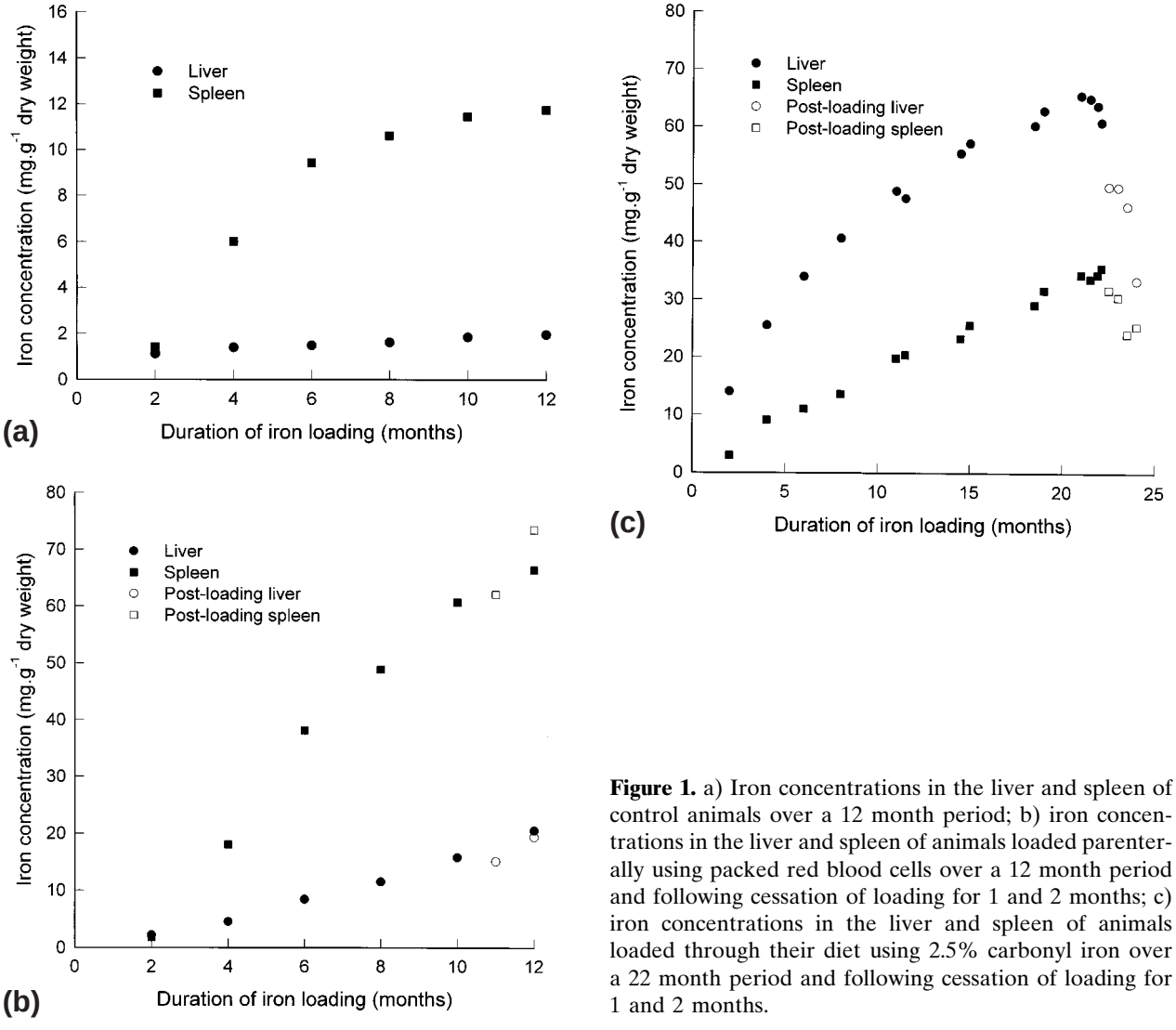


Figure 1. a) Iron concentrations in the liver and spleen of control animals over a 12 month period; b) iron concentrations in the liver and spleen of animals loaded parenterally using packed red blood cells over a 12 month period and following cessation of loading for 1 and 2 months; c) iron concentrations in the liver and spleen of animals loaded through their diet using 2.5% carbonyl iron over a 22 month period and following cessation of loading for 1 and 2 months.

area of Kupffer (non-parenchymal) cells stained blue in the same field of view were then determined. Kupffer cells were differentiated from hepatocytes on the basis of their location, size and morphology. In each case, the area staining for iron was recorded as a number of pixels, compared with the total pixels in the video display unit screen and then expressed as a percentage. In order to provide reproducible data, twenty-five non-overlapping fields, avoiding the periphery of the stained section, were measured and summated for each case. Correlations were determined using the student *t* test.

Results

General tolerance to iron loading

In general, both parenteral and dietary iron loading animals tolerated the increased iron load very well throughout the experimental period. However, some deterioration in general appearance and weight was noticed in the carbonyl iron group when compared to both the control and parenteral iron loaded animals. As mentioned earlier (in the Materials section) this became sufficiently marked in some animals that they were euthanased prior to the designated date to avoid any suffering.

Rats in the control group showed a stable liver and spleen weight of approximately 10.0 (SD ± 0.3) g and 0.7 (SD ± 0.2) g, respectively, over a period of one year. In the parenteral iron-loaded group, the weight of the liver and spleen rose from 8.4 to 14 g and from 0.9 to 1.6 g, respectively, over the one year period. In the dietary iron-loaded group, the liver weight rose from 11 to 22 g while the weight of spleen rose from 1.1 to 2.0 g over 22 months. In comparison to the weights obtained at 22 months, the weight of the liver and spleen in both groups of iron-loaded animals decreased after the cessation of loading.

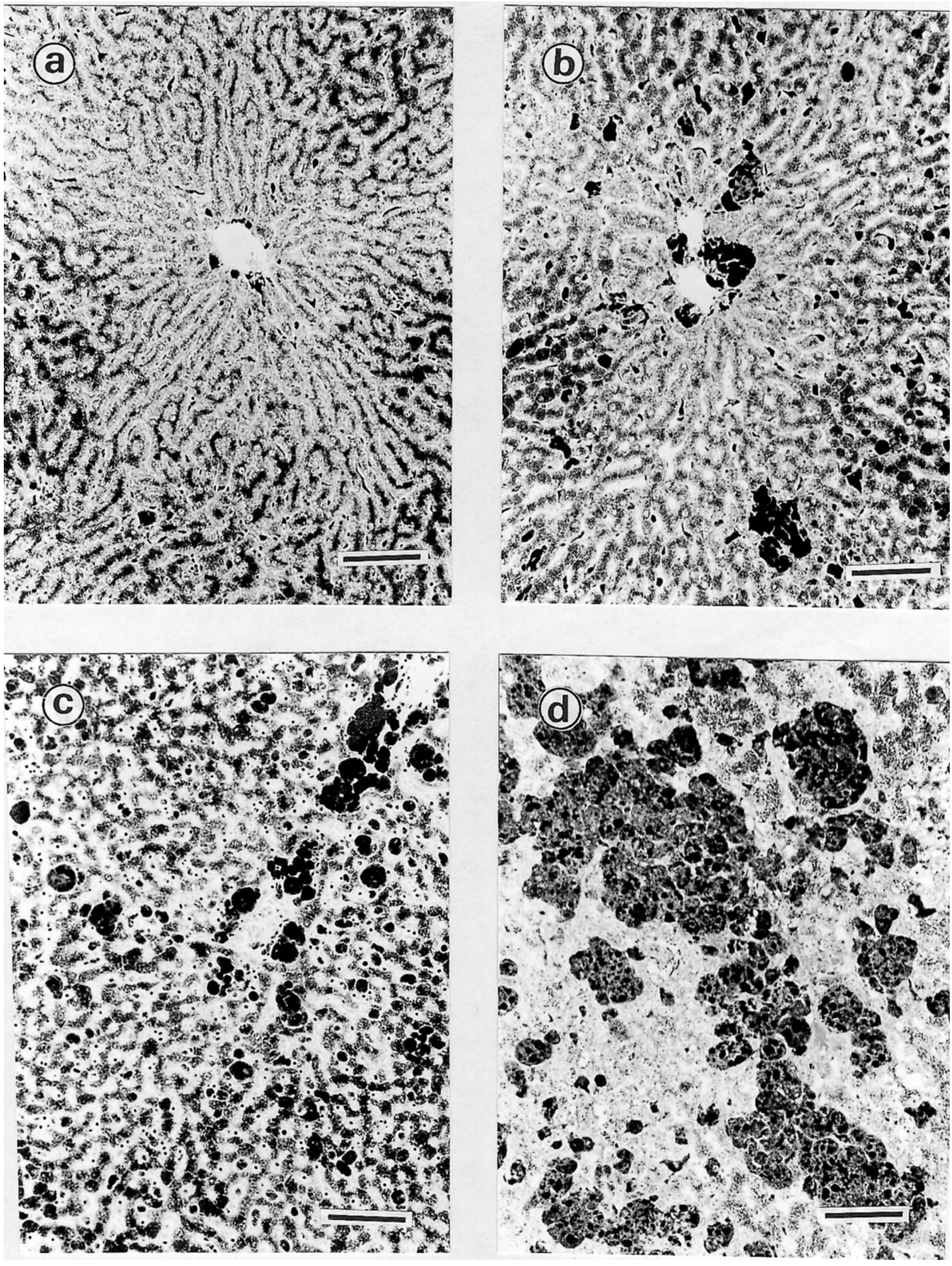
Neither the control nor the parenteral iron-loaded groups showed any sign of anaemia. In both groups, microscopic examination of the peripheral blood smears showed normochromic, normocytic red blood cells. In contrast, signs of anaemia were seen in dietary iron-loaded animals. However, there was a marked degree of variation in the level of anaemia between individual rats, as indicated by the reduction of red blood cell count, haemoglobin, and haematocrit compared with the control group. In general, the red blood cell picture from the blood smears showed hypochromic and normocytic red blood cells, corresponding to a lower mean cell haemoglobin concentration (MCHC = 344 g L^{-1}) than that found in the control (MCHC = 356 g L^{-1}) and parenteral (MCHC = 366 g L^{-1}) groups. Reticulocytosis was found in every dietary iron-loaded rat, with the reticulocyte count rising to a mean of 4.8% after 20 months of dietary iron loading compared with a mean of 0.7% obtained both from the initial control animals and the parenteral iron-loaded group. The high number of polychromatophilic (young) red cells found in blood smears of these dietary iron-loaded rats corresponded to the significantly increased reticulocyte count.

Iron concentrations in the liver and spleen

In comparison to the values found in the control animals, the concentration of iron in the liver and spleen obtained from both groups of iron-loaded animals increased dramatically with increasing age (c.f Figs. 1a, b and c). However, depending on the route of loading, marked differences occurred in the concentrations of iron found. The hepatic and splenic iron concentrations in control animals remained relatively constant, showing only a small increase over 12 months. In contrast, in parenteral iron-loaded animals, the hepatic iron concentration increased from approximately 2 mg g^{-1} dry weight at two months to approximately 21 mg g^{-1} dry weight at 12 months (Figs. 1a and b), while the hepatic iron concentration in dietary iron-loaded animals rose from approximately 14 mg g^{-1} dry weight at two months to 48 mg g^{-1} dry weight at 12 months (Figs. 1a and c). Continued loading for a further 10 months produced values in excess of 60 mg g^{-1} dry weight.

In contrast, the iron content of the spleen of control animals increased with increasing age, reaching approximately 10 mg g^{-1} dry weight after 12 months (Fig. 1a). However, this rise was very small in comparison to that which occurred in parenterally loaded animals where the iron concentration in the spleen increased from approximately 2 mg g^{-1} dry weight at two months to approximately

Figure 2. (p. 107) Light micrographs of dietary iron-loaded rat liver obtained from animals of various ages. The tissue sections were stained using Perls' reaction where the presence of iron is indicated by the dark colouration and counterstained using safranin. a) 4-month-old animal showing the periportal distribution of iron, b) 8-month-old animal showing diminished periportal gradient and appearance of iron in the Kupffer cells; c) 11 month old animal showing aggregates of iron-loaded Kupffer cells; d) 22-month-old animal showing large amount of iron in Kupffer cells distributed throughout the liver. Scale bar = 100 μm.


a
b
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66 mg g^{-1} dry weight at 12 months old (Fig. 1b). Iron concentrations in the spleen of dietary loaded animals also rose compared to that in the control animals though this was not as marked as in the parenterally loaded animals (Fig. 1c).

Cessation of iron loading, for up to two months, had relatively little effect on the iron concentration in either the liver and spleen of parenterally-loaded animals (Fig. 1b). However, in dietary loaded animals, the concentration in both the liver and spleen fell precipitously over the ensuing period (Fig. 1c).

Morphology of iron deposits

In the control animals, sacrificed at the beginning of the experimental period, no visible iron deposits could be seen in hepatocytes and only small amounts of iron were found in the Kupffer cells and in the splenic macrophages. After 12 months, control animals still had no stainable iron in the hepatocytes (grade 0) while iron in Kupffer cells was assessed as trace to mild, and in the splenic macrophages as moderate. No pathological changes were seen at the light microscopic level in either the liver or spleen in any control animal at any time during the experiment.

In the liver, dietary iron-loading initially resulted in the deposition of iron in hepatocytes in the periportal regions (Fig. 2a). However, after approximately 8 months of loading, this gradient diminished and iron deposits could be seen in all parenchymal cells and had started to appear in the Kupffer cells near the terminal hepatic vesicles (Fig. 2b). Prolonged iron loading (up to 12 months) resulted in groups of iron-loaded Kupffer cells becoming distributed throughout the liver (Fig. 2c). By 22 months, groups of iron-loaded Kupffer cells dominated the histological picture, occupying large areas of the liver, while the level of iron staining in the parenchymal cells remained stable (Fig. 2d). Two months after the cessation of iron loading, the histological picture of iron staining in both cell populations had not changed appreciably, except that the degree of parenchymal iron staining had decreased slightly. Higher magnification revealed the presence of discrete Prussian blue-stained iron granules in parenchymal cells. These granules were quite uniform in structure, being small and spherical in shape, with a peribiliary location (along the bile canaliculi), and were different in shape and size from those in the Kupffer cells. At higher levels of iron loading, the nuclei of the hepatocytes tended to be pushed to one side by the iron granules. At the same time some individual Kupffer cells appeared to have aggregated into giant cells. Visual assessment gave a numerical score of 3–4 in the hepatocytes after two months of iron loading, rising to 4 for all older animals with all hepatocytes showing marked iron deposition. Cessation of iron loading resulted in a decline in score to 3–4 after two months with some variation between individual cells. In the Kupffer cells, iron loading ranged from a trace after two months to marked after eight months. Cessation of iron loading for up to two months did not result in a decline in iron deposition in the Kupffer cells. The level of iron deposition in the spleen, while rising throughout the experimental period, was much less than that observed in the liver. Thus, visual assessment of the degree of iron loading resulted in a macrophage score ranging from a trace at two months, through to moderate/marked by the end of the loading period.

Parenteral iron loading also resulted in a dramatic increase in the total amount of iron visible in both organs but with the spleen being particularly affected. In the liver, with increased loading, fine granules of iron were again seen in the periportal hepatocytes, although at a lower density than that seen with dietary iron loading, and which eventually spread throughout the liver. In the spleen, large amounts of iron were seen in the macrophages in the red pulp and in the areas surrounding the venous sinuses (Figs. 3a and b) with very little in the follicular areas. Parenteral iron loading also resulted in the accumulation of a large number of macrophages in the spleen which also increased dramatically in size. Cessation of iron loading for two months after 10 months of iron loading did not result in any changes in the degree of iron loading. Visual assessment of the degree of iron loading in the hepatocytes resulted in scores ranging from 1 at two months to 4 at eight months. In the spleen, visual assessment of the degree of iron loading rose from mild at two months to marked at four months and remained at that level for the duration of the experimental period.

Electron microscopic studies of the liver and spleen from both groups of iron-loaded rats revealed intracellular, heterogenous electron-dense structures which were irregularly shaped and formed aggregates within membrane bound cytoplasmic bodies (Figs. 4a and b). No parenchymal damage, or abnormality other than siderosis, was seen in the liver at any stage of iron loading. Towards the end of the experimental period some collagen fibrils were visible at the electron microscope level in some livers with heavy iron loading (Fig. 4a).

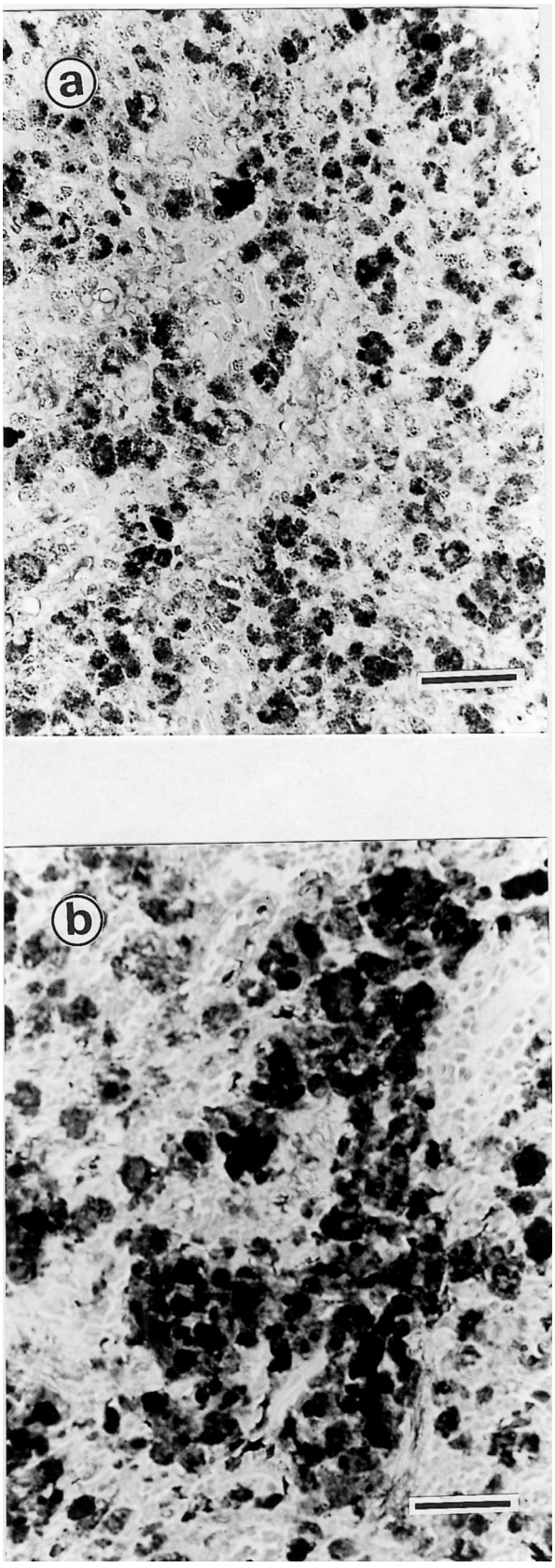

Figure 3. Light micrographs of parenterally iron loaded rat spleen taken from animals that had been iron loaded for a) 4 months and b) 12 months. Note the large amounts of iron contained within the macrophages and the tendency of these latter cells to form large aggregations, particularly in the 12-month animals. Scale bar = 100 μm.

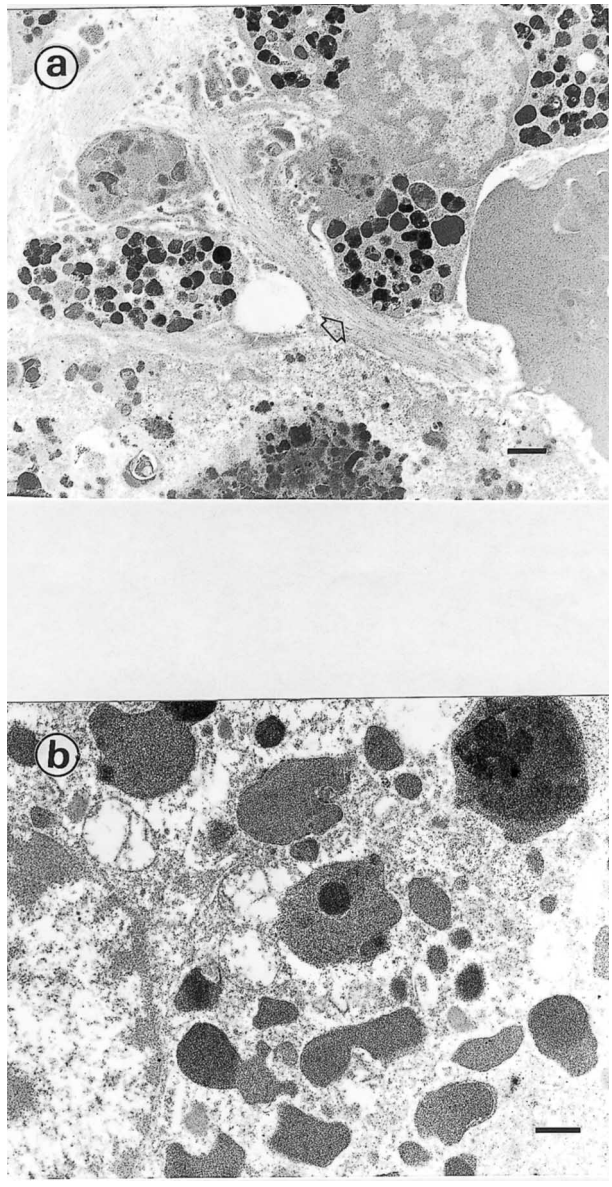

Figure 4. Transmission electron micrographs of a) rat liver following dietary iron loading for 22 months and b) rat spleen following parenteral iron loading for 12 months. In both cases note the presence of aggregations of variable electron density. Figure 4a also shows the presence of collagen fibres (open arrow). Scale bar = 1 μm.

Image analysis from the Perls' stained tissue section

Computer assisted morphometry of the liver iron concentration in both parenteral and dietary iron-loaded animals showed a high degree of correlation with the results obtained using chemical analysis for the whole liver ($r = 0.88$, $p < 0.001$; Fig. 5). Morphometric measurements of the fraction of iron in Kupffer cells also revealed a strong positive correlation with the age of the rats ($r = 0.86$, $p < 0.001$; Fig. 6) suggesting that the longer the time of loading in both iron loading groups, the greater the fraction of iron in non-parenchymal cells. In agreement with the histological iron distribution patterns, the fraction of iron in non-parenchymal cells was found to be less in the parenteral iron-loading group.

Discussion

These long term studies have shown that it is possible to load female Porton rats with dietary or parenteral iron and achieve tissue iron concentrations that are comparable to or in excess of those found in humans suffering from various forms of iron overload diseases (St. Pierre *et al.* 1998). For example, dietary iron loading for 22 months resulted in a hepatic iron concentration in excess of that found in thalassaemic patients who had received a minimal number of blood transfusions and who thus also absorbed large amounts of iron through the gastrointestinal tract (St. Pierre *et al.* 1998). Similarly, with parenteral loading the splenic iron concentration reached approximately 66 mg g^{-1} dry weight, well in excess of that found in human thalassaemic spleens where iron concentrations of up to 20 mg g^{-1} dry weight have been recorded (St. Pierre *et al.* 1998). As such, this work clearly demonstrates that carbonyl iron supplementation of the diet of rats can bypass the normal limitations on the binding capacity of the intestinal mucosa and result in significant amounts of storage iron as well as confirming the good bioavailability of carbonyl iron, although the precise mechanism of iron absorption is not clear (Nielsen *et al.* 1993). This study also confirms the previous findings of relatively low toxicity for carbonyl iron loading as suggested previously (Huebers *et al.*, 1986; Devathali *et al.*, 1991). However, despite the apparent tolerance of the rats to the iron-loading regime adopted, there were some signs of anaemia in the dietary iron group, as evidenced by the reduced haemoglobin concentration and high reticulocyte count. Indeed, this impairment of erythropoiesis may be one of the factors involved in stimulating greater iron absorption.

Factors contributing to the ability of these animals to attain high levels of iron loading may be the strain

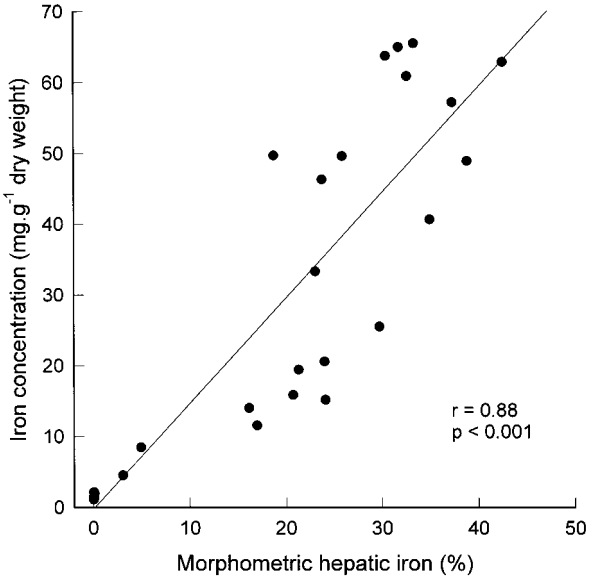

Figure 5. Correlation of hepatic iron content as determined using morphometric analysis with the liver iron concentration as determined by atomic absorption spectrometry.

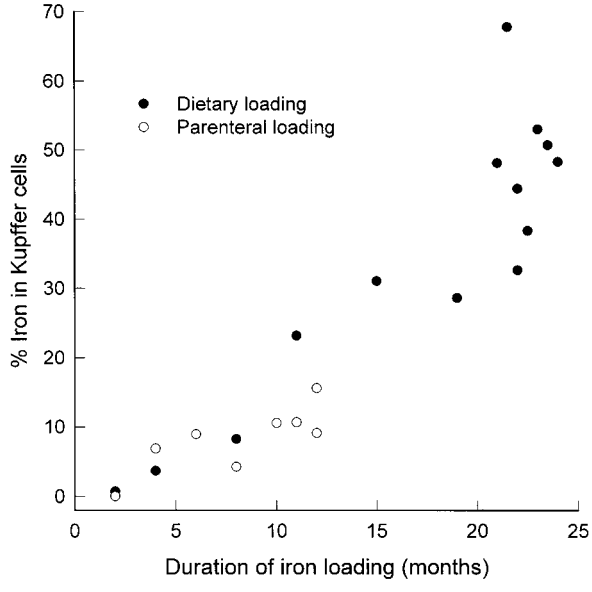

Figure 6. Correlation between the duration of iron administration and the percentage of iron in Kupffer cells, as determined by morphometric analysis for both dietary and parenterally loaded animals.

of rats used, female gender and the commencement of dietary loading virtually from birth by feeding carbonyl iron to lactating mothers (Olynyk *et al.* 1992). The high iron concentrations achieved in this study are in contrast to most of those obtained in previous investigations where prolonged feeding using carbonyl iron has not progressively increased hepatic iron loading (Park *et al.* 1987; Nielsen *et al.* 1993). For example, a similar loading regime using male Porton rats showed hepatic iron concentrations of up to 250 mmol.g^{-1} dry weight (equivalent to 14 mg g^{-1} dry weight) within 6 months (Olynyk *et al.* 1992), while in contrast, using female Wistar rats on 2.5% carbonyl iron produced a steady increase in total liver iron concentration to 2.5 mg g^{-1} wet weight after 7 weeks, with no further increase being observed up to 39 weeks (Nielsen *et al.* 1993). In another carbonyl iron study, using male Sprague-Dawley rats, it was shown the maximum concentration of iron that could be obtained was approximately 10 mgg^{-1} liver wet weight over a 4 month period with the animals remaining at this level up to 12 months of loading despite some sign of hepatocellular necrosis (Park *et al*. 1987). Only one study (Roberts *et al*. 1993), also using Sprague-Dawley animals 2.5% carbonyl iron supplemented for up to 18 months, achieved a similar high hepatic iron concentration (up to 1100 mmol g^{-1} dry weight, equivalent to 61 mg g^{-1} dry weight) with a minor degree of periportal fibrosis.

In addition to obtaining a high level of loading, these modified experimental models of iron overload in the rat (dietary and parenteral) offer the opportunity to selectively target the iron load to a specific organ, and more particularly to specific cell types. As described both biochemically and morphometrically, the liver is the predominant site for iron deposition *via* dietary loading. Iron loading through the gastro-intestinal tract results in the absorbed iron being transported *via* transferrin, and subsequently being stored preferentially in cells or organs with a high concentration of transferrin receptors at the cell surface, particularly hepatocytes (Bacon & Tavill 1984; Morgan & Baker 1986). In contrast, the spleen is the predominant site of iron storage following parenteral iron loading. When loaded *via* the parenteral route, the iron concentration of the liver also increased compared to the control group, presumably as a consequence of the phagocytosis of red blood cells by Kupffer cells. However, the major site of loading was through the reticuloendothelial cells of the spleen which in normal circumstances undertakes most erythrophagocytic activity (Ham 1974). The ability of the spleen to remove material from the blood is highly dependent on its structure, which consists of the macrophage-augmented filtration beds, interposed between arteries and veins or lymphatics. The pattern of iron loading through the spleen reflects this, with the first and highest levels of iron loading being found particularly in the red pulp where the haemosiderin-laden macrophages are mainly distributed. The increase in size of this organ is presumably a consequence of the high number of monocytes/macrophages which rapidly appear in the spleen when high levels of phagocytosis are required (Olakanmi *et al.* 1994).

The overall high level of iron loading throughout the organs examined also suggests that some redistribution of iron from one cell type, or indeed organ, to another cell type or organ can occur in this model. Evidence of iron from phagocytosed erythrocytes being redistributed or transferred from Kupffer cells to hepatocytes in the form of transferrin-bound iron or ferritin has been shown previously (Sibille *et al.* 1988). In addition, there is a possibility that ferritin might also be released from splenic macrophages following erythrophagocytosis and subsequently find its way to the liver *via* the portal circulation.

This study has also shown that there is a significant linear relationship between the amount of stainable hepatic iron measured using a microcomputer image analysis system and iron concentrations determined chemically. This finding parallels those of studies of human liver biopsies from patients suffering from genetic haemochromatosis (Deugnier *et al.* 1982; Olynyk *et al.* 1990) and confirms that the concentration of iron in at least liver and spleen tissue sections, can be assessed by using computer assisted morphometric analysis. In addition, this technique can be used to evaluate the iron content of different populations of cells.

Over the last few years it has become increasingly evident that, in humans, different conditions of iron loading and different treatment regimes result in the presence of different structural forms of iron oxide in the various organs (Mann *et al.* 1988; Dickson *et al.* 1988, Ward *et al.* 1994; St. Pierre *et al.* 1998). These forms differ in their chemical stability and thus presumably in their solubility (St. Pierre *et al.* 1998). These models for dietary and parenteral iron loading are ideally suited for further investigations into the formation of the different forms of iron oxide or the transformation of the different oxides found in biological tissues from one form into another. Any differential reactivities of these iron deposits to clinical chelators would also assist in the design of chelation regimes targeted to particular organs and particular forms of tissue iron overload.

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