Iron and the oral epithelium: a review[1]

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Body iron absorption is controlled by the duodenal mucosa which allows the intake of appropriate quantities of iron to balance, exactly, small daily losses. If these iron losses are augmented by disease, usually by chronic blood loss, or if dietary intake and absorption are impaired, negative iron balance will result. The effects of this negative balance are offset for a time by mobilization of body iron stores, but finally tissue iron is depleted, the serum iron falls, with consequent failure of iron supply to the bone marrow, and iron-deficiency anaemia results. Throughout this review the term 'iron deficiency' is used to describe the iron-deficient state in general, and when appropriate the terms 'iron depletion', 'iron deficiency without anaemia' and 'iron-deficiency anaemia' will denote more precisely the progressive stages of iron deficiency (Table 1).

Iron deficiency is widespread both in affluent and underdeveloped countries, and has claims to be the commonest deficiency disease in the world. Surveys in India and certain regions of Africa reveal that around 50% of the adult population is anaemic (Gosden & Reid 1948, Ramalingaswami & Patwardham 1949, Venkatchalam 1968). Further, in a study of anaemic Mauritian adults (WHO 1959) iron deficiency was the cause in 95% of cases.

In more affluent societies the incidence of severe iron deficiency is considerably less and more is known about the prevalence of the earlier stages of iron deficiency. Garby (1973) showed that 18–25% of Swedish women were iron-deficient, and a British survey showed that 14% of women and 3% of men in the UK had low haemoglobin levels (Kilpatrick & Hardisty 1961).

Oral epithelial abnormalities are frequent in iron-deficiency anaemia, and structural (Rennie *et al.* 1982), histochemical (Dagg *et al.* 1966), and clinical (Wray *et al.* 1975) changes may occur before significant alterations in red cell morphology or haemoglobin level are noted. In this paper the oral epithelial changes of iron deficiency will be reviewed in the light of recent developments.

Oral epithelium in iron deficiency

During negative iron balance, there is progressive depletion of iron in all tissues, and a wide range of non-erythroid changes have been described in man and animals (Dallman 1974). These have recently been reviewed by Jacobs (1982), and include nail changes, atrophic gastritis and changes in the oral epithelium. The oral changes are among the most frequent

Table 1. The stages of iron deficiency

Stage	Bone marrow iron stores	Serum iron	Transferrin saturation	Haemoglobin	Film
Iron-deficiency anaemia	Absent	Low	<16%	11 g/dl	Microcytic hypochromic
Iron deficiency without anaemia	Absent	Low	<16%	Normal	Normal
Iron depletion	Absent or reduced	Normal	Normal	Normal	Normal

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and important, and in a large series of patients studied by Beveridge *et al.* (1965) atrophic glossitis was found in 39%, angular cheilitis in 14%, and post-cricoid dysphagia (Kelly-Paterson syndrome) in about 7%. In common with other tissue changes, these may occur early even without overt iron-deficiency anaemia.

Paterson (1919) was perhaps the first to describe the microscopical appearances of the oral mucosa in anaemia. He noted atrophy of the epithelium and an apparent thinning of the underlying 'tunica propria'. Suzman (1933) and Savilahti (1946) reported histological findings from a number of anaemic patients with Kelly-Paterson syndrome. In addition to oesophageal and laryngeal changes, they described areas of atrophy, hyperkeratinization and a pronounced lymphocytic infiltrate.

A more detailed microscopic description was provided by Boddington (1959); using smears of exfoliated buccal cells from saliva, a significant reduction in cytoplasmic diameter of cells from iron-deficient subjects was found. In cases with a smooth tongue, occasional abnormally large nuclei were seen.

Using semi-quantitative techniques, Jacobs (1959) noted significant cytological staining changes in cases of 'idiopathic hypochromic anaemia'. No change of staining was noted in post-haemorrhagic anaemia, in cases of anaemia associated with leukaemia, or in pernicious anaemia. This difference was attributed to the chronicity of iron-deficiency anaemia and specifically to low tissue-iron stores, suggesting that the anaemia itself had little to do with the changes.

Monto *et al.* (1961) examined smears from the dorsal surface of tongue and from the buccal mucosa before, during and after iron therapy. Smears from untreated iron-deficient patients confirmed Boddington's (1959) finding of a reduced cytoplasmic diameter, an increased nuclear size and consequently an altered nucleocytoplasmic ratio. Monto *et al.* (1961) also noted an increased number of nucleoli, the presence of double nuclei and of bizarre nuclear patterns in untreated iron-deficient patients. These changes were reversed by iron therapy. Histological examination of lingual biopsies revealed absent papillae, thinning of the epithelium accompanied by an increased nuclear diameter and decreased cell size. The altered tissues returned to normal after iron therapy.

Jacobs (1960) examined biopsies of cheek obtained by a Wood's suction tube. There was no significant decrease in epithelial thickness, but the absence of melanin and the presence of a chronic inflammatory infiltrate were described. In the majority of cases differentiation between normal and iron-deficient biopsies was impossible on histological grounds alone, but this method of biopsy could have obscured structural or morphological abnormalities.

Although the weight of available evidence supports deficiency of iron as the prime aetiological factor in the development of the epithelial lesions, the mechanism whereby this is mediated is unclear. Jacobs (1961*a*,*b*), in a study of the epithelial changes in iron deficiency, noted decreased levels of the iron-containing enzyme cytochrome C in buccal mucosa from anaemic patients. Dagg *et al.* (1966) confirmed this finding both in patients with iron-deficiency anaemia and in those with latent iron deficiency, but no correlation between the epithelial atrophy, the symptoms, and the degree of enzyme depletion was found by either author.

In view of the lack of a clear relationship between tissue iron depletion and the development of oral epithelial changes, a role has been postulated for other essential nutrients and vitamins, especially folic acid, pyridoxine and vitamin B_{12}. Jacobs (1963), in a mainly retrospective study of severely anaemic Africans, found a very low incidence of epithelial lesions. It was suggested that iron deficiency plays a secondary or even incidental role in the aetiology of epithelial lesions in this population and a role for pyridoxine was proposed (Jacobs & Cavill 1968).

Vitale *et al.* (1966) reported the effects of an iron-deficient diet on rats given folic acid supplements and concluded that folate deficiency could be induced by dietary iron deficiency. Similarly Toskes *et al.* (1974) and Saraya *et al.* (1971) reported reduced serum folate levels in iron deficiency. In addition, a metabolic block in folate metabolism in iron-deficient subjects has been postulated, possibly due to the iron dependence of the enzyme

formimino-transferase (Vitale *et al.* 1966). In a comprehensive review of this problem, Hershko *et al.* (1975) attributed the variations in findings to poor controls and to the use of serum folate estimations. Results of their own work showed that erythrocyte folate measurements were a better indicator of folate status than serum folate and that erythrocyte folate levels were unaffected by a coexisting iron deficiency.

It has been suggested that iron is required for optimal incorporation of vitamin B_{12} into red cells (Doscherholmen *et al.* 1974). Harrison (1971) described decreased erythrocyte vitamin B_{12} levels in iron-deficiency anaemia, and iron therapy returned vitamin B_{12} levels to normal. As in the case of folate, the role of low vitamin B_{12} levels in the aetiology of the tissue manifestations of iron deficiency is uncertain.

In view of the voluminous literature reporting the epithelial manifestations of severe iron deficiency, the lack of accurate, reliable, quantitative data is surprising and has undoubtedly contributed to the conflicting views of the effects of iron deficiency upon the oral epithelium. However, the advent of stereological techniques has provided simple, accurate methods of obtaining quantitative data. Using such techniques in a controlled study, Rennie *et al.* (1982) noted a highly significant reduction in the total epithelial thickness of human buccal epithelium from patients with iron-deficiency anaemia. This decreased thickness was due to a reduction in the thickness of the middle cell layers of the epithelium, the maturation compartment.

Studies of the oral epithelium in iron-deficient animals are uncommon. In a subjective study using anaemic rats and sex-linked anaemic (SLA) mice, Drinnan (1969) found no changes in the oral epithelium when comparing severely deficient and normal animals. More recently Steele *et al.* (1981), using quantitative techniques, noted thinning of the epithelium from the anterior dorsum of the tongue in SLA mice.

Rennie & MacDonald (1982), in a stereological study of hamster ventral tongue epithelium, described a decrease in thickness of the maturation compartment and an increased thickness of the keratin layer in iron-deficiency anaemia. Changes in the proportion of the epithelium made up by the maturation and keratinized compartments were noted in iron deficiency without anaemia, and in iron depletion the progenitor compartment formed significantly more and the maturation compartment significantly less than normal.

Changes in epithelial cell size have also been reported (Rennie & MacDonald 1984*a*), with a decreased progenitor and maturation compartment cell size noted in iron-deficiency anaemia. No change was noted in the nuclear diameter suggesting an increased nucleocytoplasmic ratio in iron-deficiency anaemia. It appears that at least some of the reduction in compartment thickness must be due to a reduced epithelial cell size.

Cell kinetic studies in iron deficiency are rare. Rennie & MacDonald (1984*b*) described a reduction in the time taken for DNA synthesis (T_s) in iron-deficiency anaemia and in iron deficiency without anaemia. This reduction in T_s occurred in the presence of an unchanged labelling index, suggesting an increased cell production rate in iron-deficiency anaemia and iron deficiency without anaemia. No cell kinetic changes were noted in iron depletion. These findings suggest that as iron deficiency develops, there is an accompanying increase in the rate of new cell production but a decrease in the size of the epithelial cells.

Iron deficiency and oral cancer

In 1919 Brown-Kelly and Paterson, speaking at the same meeting, independently described the syndrome which in some countries bears their names. The syndrome, occurring mainly in women, consisted of dysphagia and post-cricoid oesophageal stricture. Associated features were longstanding anaemia, koilonychia, glossitis and fissures at the corners of the mouth. Brown-Kelly noted the occasional occurrence of carcinoma of the oesophagus, and the syndrome now includes these additional findings. Vinson (1922), whose name is associated with the same syndrome, quoted Plummer (1914) when describing a hysterical dysphagia, but noted the absence of oesophageal abnormalities and did not mention the presence of oral lesions.

The possibility of malignant change occurring in cases of Kelly-Paterson syndrome has been discussed since the syndrome was first described. Ahlbom (1936) produced evidence of a 70% incidence of oral, pharyngeal and oesophageal carcinoma in Kelly-Paterson syndrome. Simpson (1939), Videbaek (1944) and Jones (1961) all confirmed Ahlbom's findings and added that the whole of the upper alimentary tract may become 'precancerous'.

The incidence of malignant change in Kelly-Paterson syndrome seems to have a large regional variation and figures stating an incidence of 10–90% of selected populations have been quoted (Ahlbom 1936, Wynder *et al.* 1957). Some of these differences may be attributed to selection of patients and lack of consistent haematological data, but even the validity of any relationship between dysphagia and the iron-deficient state has been disputed. Thus, in a general population study, dysphagia was present in only 5% of women and no evidence of anaemia or iron deficiency was found in these cases (Elwood *et al.* 1964). On the other hand, Wright *et al.* (1968) found that all but 6 of 82 patients with oesophageal webs had evidence of current or past iron deficiency. Chisholm & Wright (1967) emphasized that, unless great care was taken, iron deficiency might be missed and that Elwood had not accounted for the possibility of iron deficiency having been present at an earlier stage.

In a recent study (Prime *et al.* 1983) in which the water-soluble carcinogen 4-nitroquinoline-oxide was painted onto the palatal epithelium of anaemic rats, the incidence of squamous cell carcinomas was found to be similar in both normal and anaemic animals, but tumour development occurred significantly earlier in iron-deficient animals (mean 183 days) compared with controls (mean 229 days). Iron-deficient animals showed a significantly greater incidence of tongue tumours than control animals.

Iron and oral *Candida albicans* infection

Several investigators have noticed an association between oral candidosis and deficiency of iron. Cawson (1963) described two patients, in a series with denture sore mouth, who had iron-deficiency anaemia and in whom candidal infection regressed following replacement therapy alone. An increased incidence of candidal infection in iron-deficient patients was noted by Fletcher *et al.* (1975). Higgs & Wells (1972) reported that patients with chronic mucocutaneous candidosis and iron deficiency had skin lesions responding to iron therapy. In 31 of their patients with mucocutaneous candidosis, 23 showed evidence of iron deficiency and iron therapy alone produced significant improvement in 9 out of 11 of these patients. They postulated that iron deficiency may result in persistent infection which is difficult to eradicate as long as the deficiency remains.

A recent animal study by Rennie *et al.* (1983) supported this view. Animals with iron-deficiency anaemia showed no difference in the incidence of experimentally-induced fungal infection when compared with normals. However, once infected, anaemic rats recovered more slowly and seemed less able to rid themselves of the fungal infection.

It is possible that the increased thickness of keratin in iron deficiency described by Rennie & MacDonald (1982) provides a more suitable environment for candidal growth, but it is difficult to understand how the epithelial abnormalities allow persistence of infection in iron-deficient animals.

Alterations in the immune response which have been described in iron deficiency could result in an inadequate host response to the fungus. Impaired lymphocyte transformation and a reduced delayed hypersensitivity reaction have been described in iron deficiency (Joynson *et al.* 1972) and such defects could result in persistence of infection.

Recently an attempt has been made to induce oral candidosis in mice with sex-linked anaemia (Sofaer *et al.* 1982). However, the use of a genetic abnormality of iron metabolism is not ideal because few genetic abnormalities of iron metabolism present in clinical practice, but this may be a useful tool for studying some aspects of iron deficiency.

In a clinical situation it is unlikely that a single nutrient deficiency is the only factor responsible for a chronic oral candidal infection, but iron deficiency appears to be important in persistent fungal infection. This association has been noticed previously (Jenkins *et al.*

1977) and it is suggested that patients with chronic oral candidal infection be routinely screened for iron deficiency.

Conclusions

There now seems little doubt that deficiency of iron has an effect upon the oral epithelium. The atrophic changes are seen in the middle cell layers of the epithelium and what evidence there is points to an alteration in the production of new epithelial cells. However, further studies are required to elucidate the mechanism of the increased cell production.

Deficiency of iron has been implicated in the aetiology of recurrent aphthous stomatitis (RAS). Although disagreement exists as to the prevalence of iron deficiency in RAS (Wray *et al.* 1975, Challacombe *et al.* 1977, Tyldesley 1983), it appears that deficiency of iron occurs in a small number of RAS patients and that these patients are improved by iron therapy.

It also appears that in experimental situations the oral mucosa in iron deficiency shows increased susceptibility to the development of intra-oral squamous cell carcinomas. This finding is compatible with the early development of hepatic neoplasia in rats in iron deficiency (Vitale *et al.* 1978).

One of the main reasons why doubt has existed about the effect of iron deficiency upon the oral epithelium has been the lack of adequate assessments of the iron status of the study group and an almost universal ignorance of the relevant vitamin levels. Too often the haemoglobin level in conjunction with the clinical appearances and perhaps an isolated serum iron level are the only investigations. Studies lacking full haematological and biochemical assays of controls and experimental groups must give inconclusive results (Ranasinghe *et al.* 1983). To provide meaningful results, studies must have a vitamin screen of at least B_{12} and folic acid of control and experimental groups. The haemoglobin level supported by a stained film and red cell indices backed up by assessment of serum iron and total iron-binding capacity or of serum ferritin will ensure a clear picture, allowing sensible conclusions to be drawn.

Other very relevant and often forgotten complicating factors which may influence the histological structure of oral epithelium are age, sex, menstruation, smoking, chewing habits and alcohol consumption. All of these have been reported as affecting the thickness of the oral epithelium.

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