THE BUCCAL MUCOSA IN ANAEMIA

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The histological features of the buccal mucosa have been examined in 50 cases of iron-deficiency anaemia, 14 cases of megaloblastic anaemia, and 50 normal subjects. Differences between the anaemic and normal groups have been found and in some anaemic patients striking morphological changes have been seen. These might be due to a direct effect of iron or vitamin B₁₂ deficiency on the metabolism of the epithelial cells.

The pathology of the buccal mucosa in anaemia is not fully understood, although symptoms related to the oral cavity, such as glossitis and stomatitis, are commonplace in severely anaemic patients. These symptoms may also be due to deficiency of iron or vitamin B₁₂ in the absence of anaemia (Waldenström, 1938; Adams, 1957).

Material

Three groups of subjects were investigated.

(1) Fifty Cases of Chronic Iron-deficiency Anaemia.—No case with a haemoglobin concentration of 9 g. per 100 ml. or higher was included. The degree of anaemia is shown in the Table.

TABLE
DEGREE OF ANAEMIA IN PATIENTS INVESTIGATED

Haemoglobin (g. per 100 ml.)	3.0- 3.9	4.0- 4.9	5.0- 5.9	6.0- 6.9	7.0- 7.9	8.0- 8.9	Total
Iron-deficiency anaemia (cases)	1	1	4	15	17	12	50
Megaloblastic anaemia (cases)	0	3	2	6	2	1	14

In 32 cases the serum iron was less than 20 µg./100 ml., and in only three cases was it greater than 50 µg./100 ml.

An estimate of the duration of anaemia was made from the patient's history. Seven had had symptoms for only a few months. Thirty-three had a history of over a year, and in some cases many years. In 10 cases no estimate could be made of the duration of the condition.

Symptoms relating to the mouth or pharynx were present in 15 patients, and koilonychia was found in four. All these patients were estimated to have been anaemic for over a year. There

was no relation between the presence of oropharyngeal symptoms and the degree of anaemia, but all these patients except one had serum iron levels below 20 µg./100 ml. Ten patients in this group complained of dysphagia, and further examination revealed the presence of a post-cricoid web in one case and a post-cricoid squamous carcinoma in another.

(2) Fourteen Cases of Megaloblastic Anaemia.—

Twelve of these patients suffered from classical Addisonian pernicious anaemia, one from Addisonian anaemia associated with hypogammaglobulinaemia, and one from multiple jejunal diverticula. The degree of anaemia is shown in the Table. Serum vitamin B₁₂ levels were estimated in the last two cases and in six of the others. The range obtained was 15–70 µg./ml. (normal 140–600 µg./ml.).

Only two patients in this group complained of oropharyngeal symptoms. Both included dysphagia amongst their complaints, but no radiological lesions were found.

(3) Fifty Non-anaemic Patients.—These patients were convalescing from minor medical or surgical conditions and apparently in good health.

None of the patients in any group suffered from a dermatological abnormality.

Methods

Specimens of the buccal mucosa were obtained by means of Wood's gastric suction biopsy tube. The orifice in the head of the tube was placed firmly but gently against the inside of the cheek between the first molars. A disc of epithelium, and usually some subepithelial tissue, about 2 mm. in diameter, was obtained and immediately fixed in

10% formol saline. The specimens were embedded in paraffin and orientated so as to obtain vertical sections through the mucosa. Sections were stained with haematoxylin and eosin. In a few instances, due either to obliquity of the section or to inadequate depth of the specimen, a full series of observations was not possible. Two biopsies were taken at different times from one patient with iron-deficiency anaemia.

The biopsy procedure is almost painless and no local anaesthetic is necessary. Most patients consider it to cause less discomfort than a finger-prick. No complications have been discovered in more than 300 biopsies.

Histological Findings

The appearance of the normal buccal epithelium is somewhat variable. Fig. 1 shows two normal specimens and illustrates the extremes of thickness. Most of the biopsies from anaemic patients cannot be clearly differentiated from the normal, but certain features have a different incidence

in the anaemic groups as a whole, and in a few cases there are obvious abnormalities. Individual characteristics of the buccal mucosa will be considered separately.

Epithelial Thickness. — Measurements were made of the maximum and minimum thickness of epithelium in each specimen. In the normal group some reduction in the maximum measurement was found in older subjects and appears to reflect a tendency for the interpapillary pegs to decrease in size with age. The maximum thickness of the epithelium fell below the normal range in nine out of 43 cases of iron-deficiency anaemia, and in three out of 14 cases of megaloblastic anaemia. In some cases thinning of the epithelium was marked (Fig. 2). The mean epithelial thickness of the anaemic groups as a whole was not significantly reduced.

Seven of the nine iron-deficient cases showing abnormal thinning had serum iron levels below $20 \mu\text{g./100 ml.}$

FIG. 1.—(a) Normal thick epithelium. (b) Normal thin epithelium. Haematoxylin and eosin $\times 100$.

FIG. 2.—Epithelial atrophy in a case of iron-deficiency anaemia. Haematoxylin and eosin $\times 100$.

	Maximum	Minimum
Normal ..	$434 \mu \pm 13.9$ (280–655)	$185 \mu \pm 9.5$ (85–350)
Iron-deficiency anaemia ..	$413 \mu \pm 25.8$ (175–780)	$186 \mu \pm 13.7$ (90–520)
Megaloblastic anaemia ..	$360 \mu \pm 29.1$ (200–610)	$199 \mu \pm 21.1$ (80–350)

Zone of Cornification.—The earliest morphological sign of cornification in the prickle cell layer of the epithelium is the sudden flattening of cells perpendicular to the surface and a tendency to pyknosis in their nuclei. The thickness of this flattened zone varies greatly between individual specimens, but there is no difference between the three groups.

	Mean Thickness
Normal ..	$40 \mu \pm 4.9$ (0–110)
Iron-deficiency anaemia ..	$38 \mu \pm 4.1$ (0–120)
Megaloblastic ..	$41 \mu \pm 6.1$ (5–90)

Keratin Formation.—Cornification of the buccal mucosa does not normally proceed to the production of fully formed keratin, and none was found in the control group of biopsies. In three

cases from the iron-deficiency group mature keratin was seen (Fig. 3), and in one there were also areas of parakeratosis (Fig. 4). All three cases had serum iron levels below $20 \mu\text{g./100 ml}$.

Keratohyaline Granules.—The relation of these granules to keratin formation is debatable. They were found in 37 of the normal group, in 40 of the iron-deficiency group, and in all the 14 cases of megaloblastic anaemia.

Basal Cell Mitoses.—The mitotic rate in the buccal epithelium varies considerably, but is higher than that found in the skin by Thuringer and Katsberg (1959), and unlike the skin there is no obvious increase in mitosis with age. The mean values for each group were:

	Mitoses per 1,000 Basal Cells
Normal ..	23 ± 2.3 (1–76)
Iron-deficiency anaemia ..	27 ± 2.9 (3–80)
Megaloblastic ..	32 ± 7.2 (2–102)

FIG. 3.—Keratin formation in a case of iron-deficiency anaemia. Haematoxylin and eosin $\times 440$.

stantia propria was noted. When present they consisted mainly of lymphocytes, but sometimes a few plasma cells or polymorphs were also seen. When they were numerous the basal layer often became somewhat disorganized and poorly defined (Fig. 2). The proportion of each group showing evidence of inflammation was:

	Subepithelial	Intra-epithelial
Normal	22%	10%
Iron-deficiency anaemia	45%	12%
Megaloblastic	43%	7%

The proportion of cases of iron-deficiency anaemia with evidence of subepithelial inflammation is significantly larger than in the normal group.

Discussion

The abnormalities of the buccal squamous epithelium which occur in iron-deficiency and the megaloblastic anaemias have been previously investigated mainly by the techniques of exfoliative cytology applied to gastric washings, saliva, or scrapings from the inside of the mouth. Graham and Rheault (1954) showed a generalized enlargement of squamous cells and their nuclei in pernicious anaemia and found some cells with giant nuclei. Binucleated cells were not uncommon. Similar changes have also been demonstrated in sprue (Gardner, 1956) and in subacute combined degeneration of the spinal cord unassociated with anaemia (Boen, Molhuysen, and Steenbergen, 1958). Boddington (1959) found that although these changes are most striking in megaloblastic anaemias they may also be seen, though to a lesser degree, in iron-deficiency anaemia. They are more common in patients with depapillation of the tongue and may sometimes be seen in patients with abnormal tongues not associated with anaemia. Jacobs (1959) has shown an increase of cornification in the squames from patients with chronic iron-deficiency anaemia.

The histological appearance of the complete oral mucosa in the anaemias has received scant attention, although the clinical picture of atrophy and thinning of the epithelium has long been recognized. Those observations which have been made are mainly from advanced cases of the "anaemia with dysphagia" syndrome. Paterson (1919), in his original description of "A Clinical Type of Dysphagia," mentions in passing that in the tongue and cheek there is a thinning of the epithelium and an infiltration of the underlying connective tissue. Suzman (1933), reporting a case of the "Paterson-Kelly" syndrome, describes thinning and keratinization of the epithelium of the

tongue and oesophagus with round cell infiltration of the submucosa at both sites. McGee and Goodwin (1938), recording a similar case, state that the oesophageal mucosa was neither atrophied nor keratinized, but there was a chronic inflammatory reaction in the submucosa. They do not refer to the oral lesions. Savilahti (1946) describes keratinization of the oral mucosa near an angular fissure in a case of anaemia with dysphagia, and states that the epithelium was thin over the tongue, pharynx, and oesophagus. In the upper oesophagus he found the cells adjoining the basal layer to be in a state of rapid division. He also notes the presence of abnormally large nuclei at this site.

Thinning of the epithelium of the tongue in a case of pernicious anaemia has been noted by Taft, Hughes, and Wood (1958).

The present cases of iron-deficiency anaemia were unselected except for the exclusion of patients with a haemoglobin level of 9.0 g. per 100 ml. or above. Some show pathological thinning of the epithelium and gross abnormalities such as keratin formation and parakeratosis, but the majority are

FIG. 6.—Squamous carcinoma of the oesophagus in a case of iron-deficiency anaemia (same case as Fig. 2). Haematoxylin and eosin $\times 70$.

not significantly different from the normal. Round-cell infiltration in the subepithelial connective tissue is common. The apparent increase in mitotic prickle cells may be a reflection of the crude methods of assessment. Oropharyngeal symptoms, such as sore tongue, sore mouth, and dysphagia, were not apparently related to the presence of any of these features. The only histological characteristic seen more commonly in patients with symptoms was a loss of melanin from the basal layer and the underlying tissue.

In the megaloblastic anaemias the interpapillary pegs also showed a tendency to atrophy, but this was not significant for the group as a whole. The presence of keratohyaline granules in all cases is perhaps surprising as mature keratin formation was not found at all. The presence of binucleate prickle cells was common in this group and loss of melanin was striking.

Depigmentation of the hair is known to occur in both pernicious and iron-deficiency anaemia, and vitiligo is also associated with these conditions. The loss of pigment from the mouth is probably part of a generalized disorder, the nature of which is unknown.

In three patients with iron-deficiency and one with pernicious anaemia complaining of dysphagia, biopsies were taken from the upper end of the oesophagus. No gross abnormality was found, but there was a mild degree of round cell infiltration under the epithelium in all the specimens. One patient with iron-deficiency anaemia who had had a sore tongue and angular stomatitis for nearly two years, and dysphagia for two months, was seen to have marked koilonychia. Biopsy of the oesophagus showed a well-differentiated squamous carcinoma arising from apparently normal epithelium (Fig. 6). The buccal epithelium, however, showed an atrophic stomatitis (Fig. 2). Cheli, Doderer, Celle, and Vassalotti (1959), who found atrophic changes in the oesophageal mucosa of seven out of 16 patients with iron-deficiency anaemia, saw no relation between this and depapillation of the tongue. It appears that not only are the pathological changes in the mouth not always reflected by the oesophageal mucosa, but that post-cricoid carcinoma does not necessarily arise as a sequel to these changes.

Conclusion

The main changes seen to occur in the buccal mucosa of some anaemic patients are a decrease in epithelial thickness and some loss of melanin pigment. Only the latter is apparently related to the presence of oral symptoms. In iron-deficiency

anaemia keratin may be formed, and in the megaloblastic anaemias binucleated cells are often seen. These abnormalities are probably the result of multiple metabolic defects in the skin which may be the sequel of iron and vitamin B₁₂ deficiency. The widely diverse processes of melanin formation, epidermal cell division, and cornification which are affected make a simple parallel between the mucous membrane changes and the bone marrow changes in anaemia (Gorz-Kardaszewicz, 1956) difficult to accept. It is unlikely that the changes observed are the direct result of anaemia, as they are not related to the haemoglobin concentration and they have been seen to occur in the absence of anaemia.

Specific deficiencies of iron-containing enzymes have been demonstrated in some tissues of iron-deficient animals (Beutler, 1957, 1959; Beutler and Blaisdell, 1960), and it is possible that similar defects in the metabolically active buccal mucosa may exist. A reduced concentration of iron in the finger-nails, an epidermal derivative, has been shown to exist in some iron-deficient patients (Jacobs and Jenkins, 1960). The part played by vitamin B₁₂ in tissue metabolism is not clear, and the possibility that some of the tissue changes following iron deficiency may be due to a secondary lack of vitamin B₁₂, such as that demonstrated by Cox, Meynell, Gaddie, and Cooke (1959), should be considered.

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