The tongue and oesophagus in iron-deficiency anaemia and the effect of iron therapy

I. McLEAN BAIRD, O. G. DODGE, F. J. PALMER, AND
R. J. WAWMAN

*From the Medical Unit, St. Helen Hospital, Barnsley, and the Department of Pathology,
University of Sheffield*

SYNOPSIS Biopsies of the tongue and oesophagus were performed on 14 patients with uncomplicated iron-deficiency anaemia before and after treatment with iron. Haemoglobin and serum iron estimations were performed at the same time.

Nine patients had clinical evidence of atrophic changes in the tongue before therapy was started. Evidence of regeneration appeared within one or two weeks of starting iron therapy. Two patients showed persistent atrophy. Angular stomatitis and koilonychia were longer in disappearing.

Biopsies confirmed that filiform papillae and kerato-hyalin granules are frequently absent from the epithelium of the smooth tongues of iron-deficient patients. Iron therapy is followed by the re-appearance of keratohyalin granules and keratinized filiform papillae.

Two patients complained of dysphagia, which disappeared after treatment. No abnormality in the oesophageal epithelium was found in any of the patients either before or after therapy. The relationship of oesophageal carcinoma to antecedent iron-deficiency epithelial changes is considered suspect.

The association of atrophy of the mucosa of the tongue and dysphagia with anaemia has been recognized for many years. The nature and relative importance of the types of deficiency responsible for this syndrome have remained obscure. Deficiency of iron, of intrinsic factor, or of other vitamins has been implicated. Even when it had become clear that glossal atrophy occurred in primary iron-deficiency anaemia, it was considered that this could be due to secondary bacterial infection or to associated vitamin deficiencies.

Dysphagia associated with iron-deficiency anaemia was described independently by Kelly (1919) and by Paterson (1919) in papers entitled respectively 'Spasm at the entrance of the oesophagus' and 'A clinical type of dysphagia'. Vinson (1922) described 69 cases in a paper entitled 'Hysterical dysphagia'. The nature of the relationship between the dysphagia and the iron deficiency state was not clearly appreciated and the dysphagia was attributed to syphilis, to neurological, or to psychological disorders.

Suzman (1933), however, described marked improvement in the oral lesions and dysphagia in six out of seven cases after treatment with iron. Walden-

ström (1938) also treated three cases with iron, and concluded: 'Under the influence of iron therapy the epithelial defects in the corners of the mouth, on the tongue, and in the throat are healed'. Scant attention has been paid to the pathology of the lingual and oesophageal lesions associated with iron-deficiency anaemia. Savilahti (1946) gave an account of the findings in an old woman with the Paterson-Kelly syndrome who died with pneumonia. The tongue showed no filiform papillae, and only a few fungiform, and the whole epithelium appeared atrophic. The oesophageal and laryngeal epithelium was described as degenerated and atrophic but with a hyperplastic basal-cell layer. An area of muscle atrophy was also described in the middle third of the oesophagus; this was thought to explain the dysphagia. Cheli, Dodero, Celle, and Vassalotti (1959) found no relation between clinical depapillation of the tongue and histological atrophy. Jacobs (1960) found that the thickness of the buccal epithelium was reduced in some anaemic patients but that the mean epithelial thickness of anaemic patients as a whole was not significantly reduced.

The present study correlates the haemoglobin and serum-iron levels with the clinical and histological

Received for publication 10 March 1961.

state of the lingual and oesophageal mucosa in patients with uncomplicated iron-deficiency anaemia, before and after treatment with iron.

METHOD OF STUDY

Fourteen patients (two male, 12 female) with iron-deficiency anaemia were selected. They all fulfilled the following conditions: (1) the initial haemoglobin level did not exceed 10 g./100 ml.; (2) blood or marrow findings were consistent with the diagnosis; (3) the serum iron was not more than 50 µg. per 100 ml.; (4) no iron therapy had been given for three months; (5) there was no clinical evidence of vitamin deficiency; (6) there was no history of recent blood loss, apart from menstruation; (7) renal disease, chronic infection, ulcerative colitis, rheumatoid arthritis, and neoplastic disease were excluded.

The first biopsies of tongue and oesophagus were taken within a week of the initial blood count. The patients then began treatment with iron, either oral ferrous gluconate, 2 g. daily, or an equivalent amount of ferrous glycine sulphate (intramuscular iron had to be given to one patient). The second biopsies were taken four weeks, and the third at three months after the start of therapy. Blood counts and serum iron estimations were performed on both these occasions.

BIOPSY TECHNIQUES

TONGUE The biopsy tube described by Taft, Hughes, and Wood (1958) is used. This consists of a knife 11 mm. long and 4 mm. wide mounted on one end of a stainless steel tube and covered with a stainless steel head in which there is a hole 2 mm. in diameter through which the biopsy is taken. The patient lies with his head supported by a pillow and, with the mouth open widely, protrudes the tongue. Wood and his colleagues anaesthetized the selected area with a submucosal injection of 2% procaine but as the biopsy is virtually painless we have found this unnecessary; an amethocaine lozenge sucked some 10 minutes before the biopsy provides adequate anaesthesia. The biopsy tube is applied lightly to the tongue with the lateral hole over the selected area and negative pressure sharply applied by an assistant by means of a 20 ml. syringe attached to the lateral tube. The knife blade is then quickly pulled down across the hole. The head of the biopsy tube is then removed and the specimen placed in 10% formol saline.

With this technique 70 biopsies have been attempted and a suitable specimen has been obtained in all cases, though with difficulty in some atrophic tongues. To obtain good results we have found it important to check that the knife blade is both sharp and correctly adjusted before biopsy. Bleeding from the biopsy site is negligible and ceases in two or three minutes. The biopsy site heals in every case without residual ulceration.

OE SOPHAGUS It is assumed that the upper end of the oesophagus is the part most likely to show histological changes in anaemia, as it is this area to which the symptom of dysphagia is usually referred and in which the post-

cricoid web occurs. The fact that mediastinitis can be a complication of trauma to the lower two-thirds of the oesophagus was another factor which persuaded us to confine our attention to the upper end of the oesophagus, which begins at about the level of the sixth cervical vertebra.

The patient fasts for 12 hours and sucks an amethocaine lozenge 10 minutes before the biopsy. The biopsy is taken with the gastric biopsy tube described by Wood (Joske, Finckh, and Wood, 1955). The metal tip, smeared with liquid paraffin, is placed on the back of the tongue and swallowed until the tip is approximately at the level of the sixth cervical vertebra. Radiological control in earlier cases confirmed the tube to be in position when the tip was 7 in. behind the incisor teeth and in subsequent biopsies we have used this measurement to confirm the tube to be in the correct position. The patient is then told to stop swallowing and while negative pressure is applied by an assistant the knife blade is quickly pulled down and a biopsy taken. The specimen is immediately placed in 10% formol saline.

Over 50 oesophageal biopsies have now been attempted and a specimen obtained on each occasion. The patient suffers no discomfort other than that caused by the presence of the tube and an occasional slight dysphagia of no more than 48 hours' duration. Most patients have been kept under observation for at least 24 hours after biopsy and none have developed mediastinitis, haematemesis, melaena, or other complication. Specimens of faeces have been examined for occult blood in several normal controls with negative results. The subject is able to enjoy a meal within two or three hours of the biopsy without any discomfort.

Occasionally the knife blade grips the mucosa without severing it completely. It is necessary to remove the tube slowly in case this occurs, to avoid excessive trauma. If the tube is found to be sticking, the knife blade must be released and a further attempt made.

CLINICAL OBSERVATIONS

All 14 patients described a rapid subjective improvement, *i.e.*, decreased lassitude and sore tongue, within a week of starting iron therapy, comparable with the subjective improvement seen after cyanocobalamin in pernicious anaemia. The signs of repapillation of the tongue, where atrophic, appeared after one to two weeks' treatment, and the tongue was fully papillated in 12 patients at the time of the third biopsy. A normal tongue with distinct papillae was easily recognizable. Complete atrophy with loss of any distinctive papillae was either total or localized to the central or peripheral zones of the tongue. After one to two weeks' iron treatment small, regenerating papillae could be seen and these have been termed 'stubby papillae'.

The rapid regeneration of papillae in the tongue contrasted with the slower disappearance of angular stomatitis. This was often still present at the time

of the second biopsy, when the tongue was normal or showing early repapillation. Koilonychia took even longer to grow out and was present in the distal half of the nail at the time of the third biopsy after three months' iron therapy. It is concluded that regeneration of lingual papillae is one of the most sensitive indices of response to iron.

Two patients showed persistent glossal atrophy after three months' therapy. In Case 9 the haemoglobin and serum iron were normal but peripheral atrophy of the tongue persisted, whereas in Case 14 there was generalized atrophy in spite of a haemoglobin of 13.4 g. %.

Two patients with anaemia had an associated dysphagia. Case 13 presented symptoms of sore tongue and dysphagia for 12 months, with a moderate anaemia (initial Hb of 7.4 g. %) and a serum Fe level of 12 μ g./100 ml. Angular stomatitis was also present. Rapid repapillation of the tongue occurred within seven to 14 days and the dysphagia had disappeared within seven days. However, by the time of the third biopsy the tongue was again sore and atrophic but without dysphagia; the haemoglobin and serum iron levels were then normal.

Case 14 had symptoms of sore tongue and dysphagia for 18 months. She was found to have 'stubby papillae', angular stomatitis, and koilonychia. The dysphagia was slower to respond and was present until eight weeks after starting oral iron. The tongue was fully papillated two months after therapy was begun. The two patients with anaemia and dysphagia had the lowest serum iron levels of the series (12 and 10 μ g./100 ml. respectively), but satisfactory treatment of both anaemia and dysphagia was achieved.

In one patient only was the response to oral iron unsatisfactory (Case 4), and a subsequent follow-up of this patient showed a chronic duodenal ulcer, which required gastrectomy.

HISTOLOGICAL OBSERVATIONS

NORMAL APPEARANCES The following description is based on the appearances of biopsies in normal subjects. The biopsies of the tongue yielded a piece of epithelium of 2 to 3 cm. diameter. The depth of the biopsy ranged from 0.8 to 1.0 mm. On only one occasion did the biopsy fail to include the full thickness of the epithelium.

The biopsies of the oesophagus provided slightly larger specimens. The diameter of the epithelium removed ranged from 2 to 4 mm., with a depth of 0.8 to 1.4 mm. Apart from a single failure, all the biopsies included the full thickness of the epithelium.

The specimens were fixed immediately in formal

saline. Orientation of the specimen for subsequent sectioning is made easier if a little methylene blue is added to the fixative just before its transfer to alcohol. Alternatively, picric acid can be used, or the specimens fixed in Bouin's fluid.

The lingual epithelium, as seen in these biopsies, varied from 15 to 40 cells in thickness. The epithelium shows alternating areas of swollen, glycogen-filled cells, and of more compact cells with more solid-looking cytoplasm. These latter areas underlie the filiform papillae, which appear as slender, stalked projections covered at their tips by a dense layer of microorganisms. The compact epithelial cells deep to the papillae show a scattering of keratohyalin (eleidin) granules. A narrow strip of the submucosal connective tissue is included in nearly all the biopsies, but no muscle fibres or salivary tissue were encountered. A typical lingual biopsy specimen taken from a healthy control is shown in Fig. 1.

The oesophageal epithelium has a thickness of 15 to 35 cells. There is a smooth transition from the columnar cells of the basal layer to the flattened cells, with their elongated pyknotic nuclei, of the superficial layers. There is no variation in the pattern such as is seen in the tongue, and the superficial layers form a smooth surface. The biopsies included part of the submucosa, and in many cases bundles of smooth muscle from the muscularis mucosae; sometimes the duct of a mucous gland, with its accompanying sheath of lymphocytes, was also included.

Fig. 2 shows the greater part of an oesophageal biopsy taken from a normal control showing the features mentioned.

APPEARANCES IN IRON DEFICIENCY AND CHANGES AFTER IRON THERAPY The tongue was normal in five patients and showed some abnormality in nine patients before treatment. In the biopsies of the nine clinically smooth tongues taken before the start of iron therapy, the epithelium showed no evidence of filiform papillae and no keratohyalin granules were present (Fig. 3). With restoration of the haemoglobin and serum iron to normal, the tongue biopsies revealed the reappearance of filiform papillae in most cases. Accompanying the papillae, scattered groups of keratohyalin granules reappeared in the underlying epithelial cells (Fig. 4). These granules only occurred at the sites where filiform papillae had formed. They were scattered throughout the depth of the Malpighian layer of the epithelium (Fig. 5). There was no correlation between the number of cell layers and the clinical and haematological state, nor was there any evidence of increased mitotic activity following iron therapy. No evidence of keratosis or parakeratosis was found.

FIG. 1

FIG. 1. *Lingual biopsy from a healthy control. Haematoxylin and eosin (× 50).*

FIG. 2. *Oesophageal biopsy showing normal epithelium, and the duct of a mucous gland with its lymphocyte sheath. Haematoxylin and eosin (× 50).*

FIG. 2

FIG. 3

FIG. 3. *Lingual biopsy showing atrophy of filiform papillae in a smooth tongue before therapy. Haematoxylin and eosin (× 210).*

FIG. 4. *Lingual biopsy showing reappearance of filiform papilla and keratohyalin granules. Haematoxylin and eosin (× 210).*

FIG. 5. *High-power view showing keratohyalin granules at the base of a filiform papilla. Haematoxylin and eosin (× 600).*

FIG. 4

FIG. 5

Oesophagus We have failed to observe any consistent histological change in the oesophageal epithelium as seen in biopsies of our patients before and after iron therapy. Counts of cell layers and of mitoses have shown no significant changes. The nature of the biopsies does not of course permit observation of the oesophageal muscle coats. Fragments of muscle tissue were, however, present in some biopsies and appeared histologically normal.

The only abnormal epithelial pattern was seen in Case 11 (second biopsy), which showed patchy areas of abnormal maturation, the superficial layers instead of being flattened, retained the columnar form of the basal layer so that the surface of the mucosa was very irregular. The third biopsy of this case, however, showed a normal pattern. There was no dysphagia in this case.

DISCUSSION

The smooth appearance of the tongue seen in some cases of iron-deficiency anaemia is due to the disappearance of the filiform papillae. When iron therapy is given, new papillae are evident clinically after one to two weeks, and biopsy after one month's therapy has confirmed their reappearance. The lingual changes are a sensitive index of iron repletion and the tongue returns to normal long before angular stomatitis and koilonychia have disappeared. The tongue biopsies reveal that there is no evidence of increased regenerative activity in the basal-cell layers of the epithelium following iron therapy. There was considerable variation in the numbers of filiform papillae per high-power field in the iron-deficiency cases before therapy. However, the mean number of filiform papillae was more than doubled after the iron store had become replete. Our findings show a correlation between filiform papillae and iron repletion.

The return to normal is marked by the appearance of foci of keratohyalin granules in the Malpighian cells and the formation of keratinous papillae at these sites. Keratohyalin granules are known to be constantly associated with keratin formation, though whether they are concerned in its synthesis or represent a degeneration product, is uncertain. They are rich in calcium mucopolysaccharides and alkaline phosphatase (Lorincz and Stoughton, 1958) but contain no detectable iron. Keratin itself contains large amounts of cystine. Chemical analysis of nail clippings in cases of koilonychia associated with hookworm infestation (Jalili and Al-Kassab, 1959) has shown that the cystine content of the nail keratin is reduced in this condition. It may be that there is a relationship between body iron levels and the metabolism of sulphur-containing amino-acids. Both koilonychia and glossal atrophy may, however,

occur without overt evidence of iron deficiency, and the relationship is clearly not a simple one. Beutler (1957) has suggested that the symptoms of iron deficiency may be related to disturbances of tissue metabolism rather than simply to a lowered haemoglobin level. The body iron stores may be depleted even though the blood haemoglobin is normal.

Beutler believes that in patients with latent iron-deficiency anaemia there may be a deficiency of other iron-containing respiratory intermediates. In rats depleted of iron by repeated bleeding he found that the amount of cytochrome C in the liver and kidneys fell before there was a drop in the haemoglobin. This work casts doubt on the claims of Hahn and Whipple (1936) that the iron-containing intracellular respiratory pigments are maintained inviolate in iron-deficiency states at the expense of haemoglobin. Low serum cyanocobalamin (vitamin B₁₂) levels have recently been reported in iron-deficiency anaemia (Cox, Meynell, Gaddie, and Cooke, 1959). At present it can only be speculated that the link between iron metabolism and keratin synthesis may be found in the iron-containing pigments concerned with intracellular respiration. Present-day histochemical techniques are not sufficiently advanced to permit estimation of the content of respiratory pigments and enzymes of individual cells. Jacobs (1960) found three patients with iron-deficiency anaemia who showed keratinization and parakeratosis of the buccal mucosa.

In our present series of lingual biopsies there appears to be a close correlation between histological appearances and the clinical description of atrophy. Although some tongues which appear clinically to be normal showed only a few filiform papillae, none of the atrophic tongues had more than one filiform papilla per high-power field.

The Paterson-Kelly syndrome is now a rarity in Great Britain. Only two cases were found in over 18 months' search among cases of iron-deficiency anaemia (Cases 13 and 14). The biopsy findings in the present cases did not confirm the hyperkeratinization and atrophic change reported by Suzman (1933) and Savilahti (1946) at necropsy. There was no evidence of any epithelial lesion in the oesophagus in our patients (with or without dysphagia). Neural or muscular abnormality could not, however, be excluded, since the biopsies only reached the submucosa in most cases.

Two patients complained of dysphagia which disappeared after treatment. No abnormality in the oesophageal epithelium was found in any of the patients either before or after therapy. This finding has recently been confirmed by Jacobs (1960) who noted a patient with postcricoid carcinoma arising from a normal epithelium. It is clear that changes in

the oesophageal epithelium do not accompany glossal atrophy and the relationship of post-cricoid carcinoma to possible oesophageal hyperkeratinization in iron-deficiency anaemia is doubtful.

We would like to thank Dr. D. E. Price, consultant pathologist, Barnsley Hospitals for cooperation. Great help and encouragement throughout this work is acknowledged to Professor D. H. Collins, Department of Pathology, University of Sheffield, and to the staff of this department.

REFERENCES

Beutler, E. (1957). *Amer. J. med., Sci.*, **234**, 517.

- Cheli, R., Doderio, M., Celle, G., and Vassalotti, M. (1959). *Acta Haemat. (Basel)*, **22**, 1.
- Cox, E. V., Meynell, M. J., Gaddie, R., and Cooke, W. T. (1959). *Lancet*, **2**, 998.
- Hahn, P. F., and Whipple, G. H. (1936). *Amer. J. med. Sci.*, **191**, 24.
- Jacobs, A. (1960). *J. clin. Path.*, **13**, 463.
- Jalili, M. A., and Al-Kassab, S. (1959). *Lancet*, **2**, 108.
- Joske, R. A., Finckh, E. S., and Wood, I. J. (1955). *Quart. J. Med.*, **24**, 269.
- Kelly, A. B. (1919). *J. Laryng.*, **34**, 285.
- Lorincz, A. L., and Stoughton, R. B. (1958). *Physiol. Rev.*, **38**, 481.
- Paterson, D. R. (1919). *J. Laryng.*, **34**, 289.
- Savilahti, M. (1946). *Acta med. scand.*, **125**, 40.
- Suzman, M. M. (1933). *Arch. intern. Med.*, **51**, 1.
- Taft, L. I., Hughes, A., and Wood, I. J. (1958). *Lancet*, **2**, 69.
- Vinson, P. P. (1922). *Minn. Med.*, **5**, 107.
- Waldenström, J. (1938). *Acta med. scand., Suppl.* **90**, p. 380.