ULTRASTRUCTURAL LOCALISATION OF IRON IN THE JEJUNUM OF BLACK CHILDREN WITH PELLAGRA

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Objective. To elucidate the pathogenesis of iron overload in the jejunum of black children with pellagra by means of comparative ultrastructural studies of iron deposition in the jejunal enterocytes of these children and adult blacks with dietary iron overload (DIO).

Design. Retrospective study of jejunal biopsies from 10 black children (age 4 - 10 years) with pellagra.

Methods. Jejunal biopsies were subjected to routine transmission electron microscopy, which enables reliable structural identification of ferritin and haemosiderin.

Results. In all the pellagra patients, deposits of ferritin and/or haemosiderin were found in the jejunal absorptive cells in 4 intracellular localisations, viz. apical vesicles, dense bodies (siderosomes), Golgi complex and basolateral areas of the cell. This pattern of iron deposition in enterocytes of black children with pellagra was similar to that previously found in adult blacks with DIO. However, in sharp contrast to DIO, no excessive iron deposition could be demonstrated in the intercellular space and lamina propria of the malnourished children.

Conclusion. These results suggest that the iron overload in the jejunal absorptive cells of black children with pellagra may be due to a defect in the release of iron from these cells.

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Increased intestinal absorption of iron is an essential feature of the dietary iron overload (DIO) found in the indigenous adult, black populations of sub-Saharan Africa.[1] This DIO syndrome is mainly attributed to high concentrations of bio-available iron in the traditional home-brewed beer consumed, especially in rural areas.[2,3] The ultrastructural features of iron deposition in the intestinal epithelium of adult blacks with histologically proven DIO of the liver have been described elsewhere.[4] In the present report we wish to illustrate the occurrence of iron-containing proteins, ferritin and haemosiderin, in the jejunal epithelial cells of black children with pellagra as well as to draw attention to close similarities in the localisation and types of iron compounds in these patients and adults with DIO.

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PATIENTS AND METHODS

During 1968/70 jejunal biopsies were obtained with a Crosby capsule from 16 black children with pellagra. All the patients were between the ages of 4 and 10 years and biopsies were collected during the first 4 days after admission to hospital. The clinical features of these patients have been described,[5] and a short abstract on the ultrastructural findings has been published.[6]

Recent re-examination of thin sections revealed that tissues from 10 of the original 16 patients showed adequate ultrastructural preservation; tissue blocks of these 10 patients form the basis of this report. It must be emphasised that the fixation (glutaraldehyde/osmiumtetroxide), processing and embedment (epon/araldite) procedures applied to tissues of the pellagra children were similar to those used in our studies on adult blacks with DIO.[4]

Application of conventional electron microscopy, as used in our studies, enables one to reliably identify two of the iron-containing proteins involved in iron absorption, namely ferritin and haemosiderin.[7-10] Briefly, ferritin appears as well-demarcated dense granules approximately 5 nm in diameter, while haemosiderin forms larger granules (diameter 8 - 10 nm) with greater variation in size and with 'fuzzy' borders. When coalescence of haemosiderin granules occurs, extremely dense granular deposits are formed.

RESULTS

In the intestinal epithelial cells of all pellagra patients, electron-dense granular deposits were found in four intracellular localisations: (*i*) in apical vesicles in the terminal web and adjacent areas of the apical cytoplasm (Fig. 1, single arrows); (*ii*) in round or bizarre-shaped dense bodies that often contained heterogeneous deposits (Fig. 1, double arrows); (*iii*) in the Golgi complex (Fig. 1, G); and (*iv*) in the basolateral areas of the cell, characteristic ferritin molecules were seen free in the cytoplasm adjacent to the basolateral cell membrane and within ferritin-containing bodies (Fig. 2D).

Ferritin granules were seen in some apical vesicles (Fig. 2A, circle), while others contained haemosiderin (Fig. 2A, arrow).

The round or oval dense bodies had a finely granular matrix and contained numerous ferritin granules (Fig. 2C), while the bizarre-shaped bodies showed abundant haemosiderin granules (Fig. 2B, H). As first noted by Richter,[9] these 'siderosomes' also contained non-iron deposits, e.g. lipid (Fig. 2B, L). In our studies of adult blacks with DIO[4] we found that

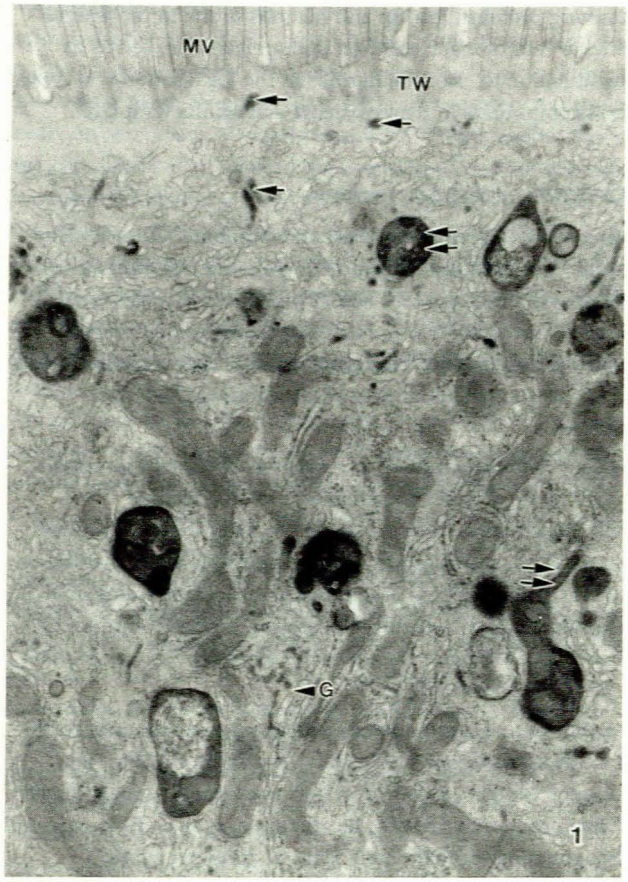

Fig. 1. Jejunal epithelial cell: electron-dense granular deposits are localised in apical vesicles (single arrows) within the terminal web (TW) or adjacent cytoplasm, in round or bizarre-shaped dense bodies (double arrows), and in the Golgi complex (G) microvilli (MV) (×120 000).

these haemosiderin-containing structures were acid-phosphatase-positive and were therefore secondary lysosomes.

Although the Golgi complex in pellagra patients occasionally appeared small and 'inactive' (Fig. 1), the usual appearance is illustrated in Fig. 2E, i.e. dilated Golgi cisternae with Golgi vesicles filled with extremely dense haemosiderin granules (Fig. 2E, arrows). Haemosiderin- and lipid-laden siderosomes were found adjacent to the complex (Fig. 2E, S).

Ferritin molecules were seen in the basal area of the cytoplasm adjacent to the nucleus (Fig. 2D). The ferritin occurred in ferritin bodies similar to those found in the supranuclear cytoplasm and as free ferritin in the cytoplasm clustered adjacent to the basolateral cell membrane (Fig. 2D).

In the pellagra children we could not find clear evidence of ferritin or haemosiderin in the intercellular space (Fig. 2D) or in the cells of the lamina propria (Fig. 3A). This was in sharp contrast to the lamina propria of adult patients with DIO, which showed macrophages filled with large ferritin- and haemosiderin-containing bodies (Fig 3B).

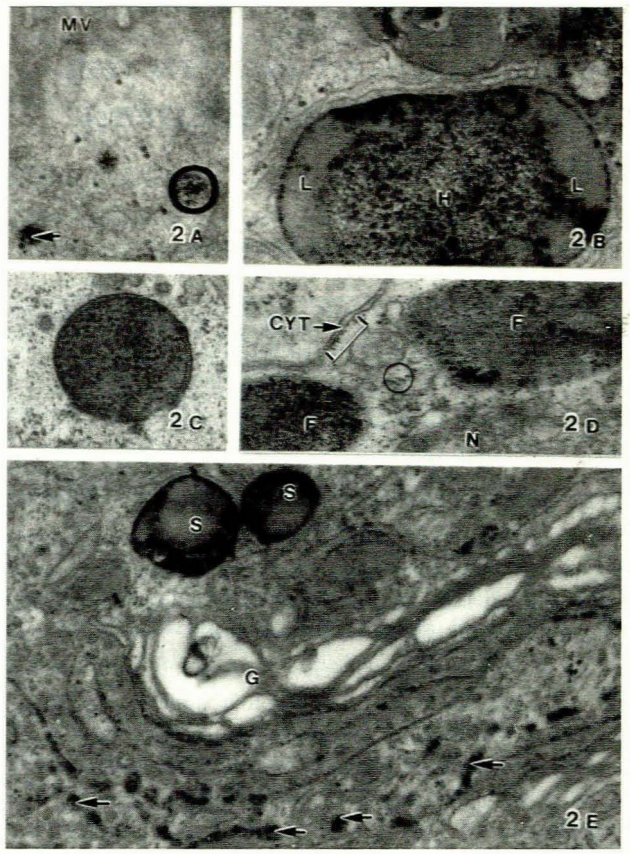

Fig. 2A. Apical vesicles that contain either ferritin (circle) or larger haemosiderin granules (single arrow) are shown in the microvilli (MV) (×42 000).
Fig. 2B. A dense body contains haemosiderin (H) and lipid (L) deposits (×42 000).
Fig. 2C. A typical round dense body is delineated by a unit-membrane and contains ferritin granules approximately 5 nm in diameter (×120 000).
Fig. 2D. The basolateral cytoplasm of an enterocyte adjacent to the cell nucleus (N) shows two bodies (F) with finely granular matrix and numerous electron-dense ferritin molecules. Ferritin is also seen free in the cytoplasm (e.g. in circle) and aligned against the cell membrane (e.g. in demarcated area). The cytoplasm of an adjacent cell (CYT) in the lamina propria and the intercellular space (single arrow) appear free of ferritin (×30 000).
Fig. 2E. Dilated cisternae of an 'active' Golgi complex (G) are illustrated with haemosiderin-containing Golgi vesicles (single arrows), while haemosiderin- and lipid-containing siderosomes (S) are found adjacent to the complex (×35 000).

DISCUSSION

The pattern of iron deposition in the jejunal absorptive cells of black children with pellagra was similar to that previously described in adult blacks with secondary DIO.[4] This not only applies to the type of iron-containing proteins (ferritin and haemosiderin) found in these two groups, but also to the organelles in which excessive intracellular iron proteins were found (i.e. apical vesicles, ferritin-haemosiderin-containing dense bodies and vesicles of the Golgi complex). Structural evidence of iron overload of jejunal epithelial cells was never encountered in other common diseases of malnutrition in black

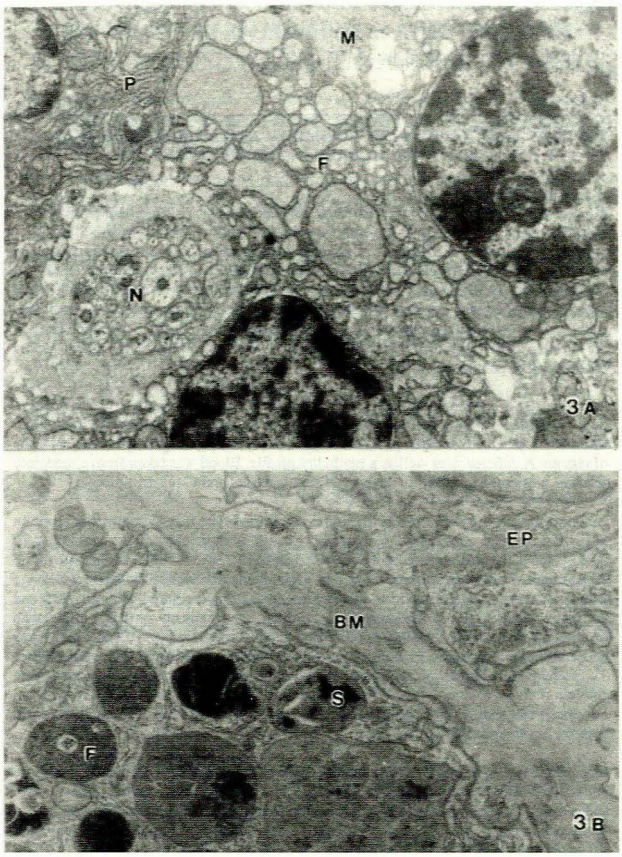

Fig. 3A. Cells in the lamina propria of a pellagra patient (P = plasma cell; F = fibroblast; M = macrophage; N = myenteric axons) appear free of ferritin and/or haemosiderin (×30 000). Fig. 3B. A macrophage in the lamina propria of an adult black with dietary iron overload shows ferritin bodies (F) and haemosiderin-containing siderosomes, some with typical cholesterol clefts (S) (BM = basement membrane; EP = epithelial cell) (×30 000).

children, e.g. kwashiorkor.[11,12]

The pathogenesis of the iron overload in the jejunum of children with pellagra is not clear. The staple diet of our patients was maize in the form of thin gruel in the early post-weaning years and more solid maize porridge in later years.[5] It is generally accepted that iron is poorly absorbed from maize.[13] In adult patients who develop DIO associated with consumption of maize/sorghum beer, three factors are responsible for enhanced iron absorption, namely removal of solids during fermentation, and the presence of ethanol and lactic acid in the final brew.[14] There is no evidence that any of these factors could be applied to food preparation for our patients. There was no overt clinical evidence of ascorbic acid deficiency in these patients. However, the clinical absence of scurvy is not sufficient to rule out tissue ascorbic acid deficiency, which may alter iron metabolism significantly.[15] The predominant vitamin deficiency was niacin, as evidenced by the rapid clearance of the pellagra dermatitis after niacin treatment.[5] Routine haematological tests were done on all patients, but anaemia was not diagnosed in the 10 patients described in this report (J G Prinsloo — personal communication).

The most striking difference between pellagra children and adults with DIO, namely the pattern of iron distribution in the basal cytoplasm and lamina propria, may be indicative of a defect in intestinal handling of iron in the malnourished children.

As described above, molecules of ferritin iron were seen free in the cytoplasm and within numerous ferritin-containing bodies in the basolateral areas of the jejunal absorptive cells of pellagra patients (Fig. 2D). However, in these patients no ferritin or other iron-containing proteins could be demonstrated in the intercellular space or in the lamina propria (Fig. 3A). This is in sharp contrast to the occurrence of ferritin in the intercellular space and of ferritin-haemosiderin within cells of the lamina propria of adult blacks with DIO (Fig. 3B).[4] Since the final step in the absorptive process is the transfer of iron across the basolateral surface of mucosal cells into the portal circulation,[16] this difference in iron localisation between pellagra patients and adults with DIO suggests a defect in the release of ultrastructurally demonstrable iron from jejunal enterocytes in the malnourished children. The mechanism of this transfer is poorly understood.[16] In *in vitro* studies, Snape and Simpson[17] recently found that low-molecular-weight serum components such as bicarbonate may mediate iron transfer from the basolateral membrane to serum transferrin. Although our structural studies do not provide an answer, it is tempting to suggest that a deficiency in a serum component may be involved in the block in iron release in the jejunum of young children with pellagra.

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