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INTESTINAL EFFECTS OF IRON DEFICIENCY ANEMIA IN CHILDREN*

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SUMMARY: Ercan O, Ulukutlu L, Özbay G, Arda O. (Departments of Pediatrics, Pathological Anatomy, and Histology and Embryology, Istanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Intestinal effects of iron deficiency anemia in children). Turk J Pediatr 33: 85-98, 1991.

A group of 11 children with iron deficiency anemia were studied with respect to intestinal structure and function. In six cases there were histological abnormalities of intestinal mucosa in varying degrees consisting of villous damage, increased activity in the crypts, increased lymphoplasmocytic infiltration and changes in the surface epithelium. Ultrastructurally, microvilli lesions, mitochondrial changes and an increase in lysosomes were observed. Relative malabsorption of iron and d-xylose malabsorption were present in a minority of patients. Functional and structural changes were correlated. Our results suggest that these changes are due to impairment of cell metabolism. *Key words: iron deficiency anemia, intestinal mucosa, mitochondria, d-xylose malabsorption.*

Many compounds in the body known to perform a metabolic or enzymatic function, contain iron[1]. Iron deficiency that has progressed beyond the depletion of iron stores affects tissue iron compounds and these effects have distinctive intracellular and organ distributions that are determined by a variety of factors[2]. It has been demonstrated that in the growing rat the tissues that are most resistant to iron deficiency are those which are fully grown and those which are subjected to a continual obligatory work load, especially of a nature that is essential for survival, like the heart muscle[1]. On the other hand, epithelial cells are constantly renewed, and iron enters these cells when they are produced[3]. Thus, in iron deficiency, one might expect abnormalities of the intestinal mucosa, which are an example of epithelial tissues with a rapid turnover. It is known that in children there is impairment in the absorptive function of the intestinal mucosa associated with iron deficiency[1]. Certain investigators have described morphological changes occurring in the intestinal mucosa[4,5], while others, have found the intestinal

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mucosa morphologically normal[6], and still others have described these morphological changes as insignificant[7].

The present study was undertaken to examine the intestinal mucosa morphologically and functionally in iron deficiency anemia. In previous studies, iron-dependent cytochrome oxidase activity in experimental animals and in children was determined so as to define the precise mechanism by which iron deficiency results in defects in intestinal function[8,9]. However, our aim was to examine the ultrastructure of the intestinal mucosa which had not been previously investigated in iron deficiency.

Material and Methods

The study group consisted of 11 patients hospitalized in the Pediatrics Department of the Istanbul University Cerrahpaşa Faculty of Medicine, between 1983-1985 with the diagnosis of iron deficiency anemia. None of the subjects had a history of infections or chronic disease. There were four females and seven males who ranged in age from one to 12 10/12 years (mean age: 5 9/12 ± 4 8/12 years). One child (Case 1), who was a twin, had a diet which consisted only of cow's milk. Another child (Case 3) had positive guaiac stool tests on four occasions and was operated on later because of a fibromyoma of the jejunum. The other subjects did not receive a balanced diet. There was a history of pica in three patients: Cases 4 and 9 ate earth, and Case 5 ate earth, sand and chalk.

Laboratory studies included hemoglobin, hematocrit, reticulocyte, leukocyte and platelet counts, examination of serum iron, total iron-binding capacity (TIBC), transferrin, osmotic fragility, Hb F, Hb A_2, erythrocyte sedimentation rate, total serum protein, albumin, globulin, liver function tests, and examination of the feces for parasites. Blood smear erythrocyte morphology was evaluated and a differential leukocyte count was performed. Bone marrow aspirates were examined. Mean corpuscular hemoglobin concentration and the serum transferrin saturation level were calculated. Intestinal biopsies were taken from all patients by means of a pediatric Watson biopsy capsule. Light microscopy revealed changes in the villi, crypt zone, surface epithelium, and lamina propria which were graded according to the following criteria: (Grade I) slight shortening and broadening of the villi, slight thickening of the basal layer, increased mitotic activity in the crypt zone, slight compaction of surface enterocytes, and a slight increase in the cellularity of the lamina propria; (Grade II) obvious shortening and thickening of the villi, thickened basal layer and crypt hyperplasia, squat, disordered and more compact enterocytes, an increase in the number of intra-epithelial lymphocytes and the cellularity of the lamia propria is greater; (Grade III) excessive shortening of the villi, mucosal flattening, obvious compaction and disorder of surface enterocytes, obvious thickening of the basal layer,

crypt hyperplasia, an increase in the number of intra-epithelial lymphocytes, and an abundance of cells in the lamina propria.

A part of the specimen was examined in six patients using the Carl Zeiss (EM 10) electron microscope and electron micrographs were obtained. In one patient, the ultrastructure was re-examined.

An oral test dose of 0.5 g/kg d-xylose was used in five patients. At 30-60 minutes after the administration of this test, d-xylose levels of 30-50 mg/dl are normally reached[10]. Since the d-xylose levels in the other five patients were low, 5 grams of oral d-xylose was given to these subjects by the method of Rolles et al[11]. According to this method, a blood d-xylose level exceeding 20 mg/dl (35 mg/dl on average) is usually reached at 60 minutes.

Iron absorption was investigated by an oral iron absorption test. After an overnight fast, blood was collected for a fasting serum iron and ferrous fumarate (65 mg per tablet) was given. Patients weighing 12.5 kg or less received one tablet of ferrous fumarate, patients weighing 12.5-20 kg received two tablets, and patients weighing over 20 kg received three tablets. Blood was drawn at one and three hours following the oral iron test dose. The resulting absorption curves were described as being "elevated" or "flat", depending on whether the maximum rise over baseline values in the serum iron concentrations at either one or three hours was greater or less than 100 μg/dl, respectively.

The Mann-Whitney *U* test and linear correlation were used for statistical analysis.

Results

Some of the hematological and biochemical data are summarized in Table I. Two children had elevated reticulocyte counts: one (Case 8) had a transferrin saturation level of 2.0 percent. TIBC was not determined in the other child (Case 2) but Hb A_2 and Hb F values were normal. The chief findings on the blood smear were hypochromia, microcytosis, anisocytosis and poikilocytosis. Neither patient had parasites.

Intestinal biopsy findings (Tables II and III) : A satisfactory biopsy was obtained in all patients. In two biopsy specimens, the only pathological finding was edema of the lamina propria (Cases 7 and 11). Six of the specimens showed the following histological abnormalities under light microscopy: shortening and broadening of the villi, activity in the crypts, lymphoplasmocytic infiltration, eosinophils and edema in the lamina propria were increased and changes in the surface epithelium consisting of a decrease in height, disorder and an increase in the intraepithelial lymphocytes. Three biopsy specimens showed Grade I changes, two showed Grade II changes (Fig. 1) and one showed Grade III changes (Fig. 2).

These findings are documented in Table II. Under the electron microscope the main pathological findings of the surface epithelium were disorder and rarefaction of the microvilli, changes in the mitochondria and an increase in lysosomes. A microvillus lesion, along with a loss of surface coating fuzz (Fig. 3), were observed

TABLE I: Hematological and Biochemical Data in Patients With Iron Deficiency

Case No.	Age (Yrs)	Sex	Hb g/dl	Hct 1/1	Ret $x10^9/1$	Leukoc $x10^9/1$	Thr $x10^9/1$	Serum İron µg/dl	TIBC µg/dl	Transferrin saturation %	Transferrin mg/dl
1	1	M	7.2	0.22	110	10.3	210	41.0	503	8.1	865
2	1 6/12	N	5.6	0.17	410	4.8	170	23.3			700
3	7 7/12	F	4.8	0.16	140	4.6	200	6.8	431	1.6	
4	11 4/12	H	9.0	0.27	50	3.2	190	3.0	635.8	0.5	
5	4	M	5.7	0.20	130	3.2	140	26.0	305	8.5	530
6	1 3/12	M	8.8	0.26	60	8.2	380	7.0	426	2.0	405
7	1 3/12	M	9.0	0.26	270	5.1	280	15.3	669	2.2	515
8	10 2/12	F	6.4	0.20	590	4.9	240	25.0	328	7.62	335
9	10	F	8.0	0.22	140	2.8	220	18.0	630	2.0	350
10	12 10/12	M	6.0	0.18	130	3.0	200	25.0	275	9.0	450
11	2	M	7.0	0.21	140	3.4	200	25.0	381	6.56	415
Mean	5 9/12		7.0	0.21	197	4.8	220.9	19.58	458.38	4.8	507.2
SD	4 8/12		1.5	0.04	165	2.2	64	11.0	145	3.4	174.0

Ret : reticulocyte
Leukoc: leukocyte
Thr : Thrombocyte
TIBC : Total iron - binding capacity

TABLE II: Intestinal Biopsy Findings in Patients with İron Deficiency

	VILLI				SURFACE EPITHELIUM			LAMINA PROPRIA		
Case No.	Height	Broadening	Grade of Villous Change	Increase Activity in Crypts	Height	Disorder	Intraepithelial Lymphocytes	Edema	Lymphoplasmocytic Infiltration	Eosinophil Number
1	↓	+	I	+	N	+	↑	++	↑	↑
2	N	+	I	+	N	+	↑	+	↑↑	N
3	N	–	0	–	N	–	N	–	N	N
4	↓	+++	II	++	↓	–	↑	+	↑↑	N
5	N	+	I	++	N	+	N	++	↑↑	N
6	N	N	0	–	N	–	N	++	N	N
7	N	N	0	–	N	–	N	++	N	N
8	↓	++	II	++	↓↓	++	↑↑	–	↑↑	N
9	Flat mucosa		III	+++	↓↓↓	–	↑↑		↑↑↑	↑
10	N	–	0	–	N	–	N	–	N	N
11	N	–	0	–	N	–	N	+	N	N

Fig. 1 (Case 4): Grade II villous damage: villi are broader and shorter, crypt zone hyperplastic. Lymphoplasmocytic infiltration increased in the lamina propria (H+Ex80).

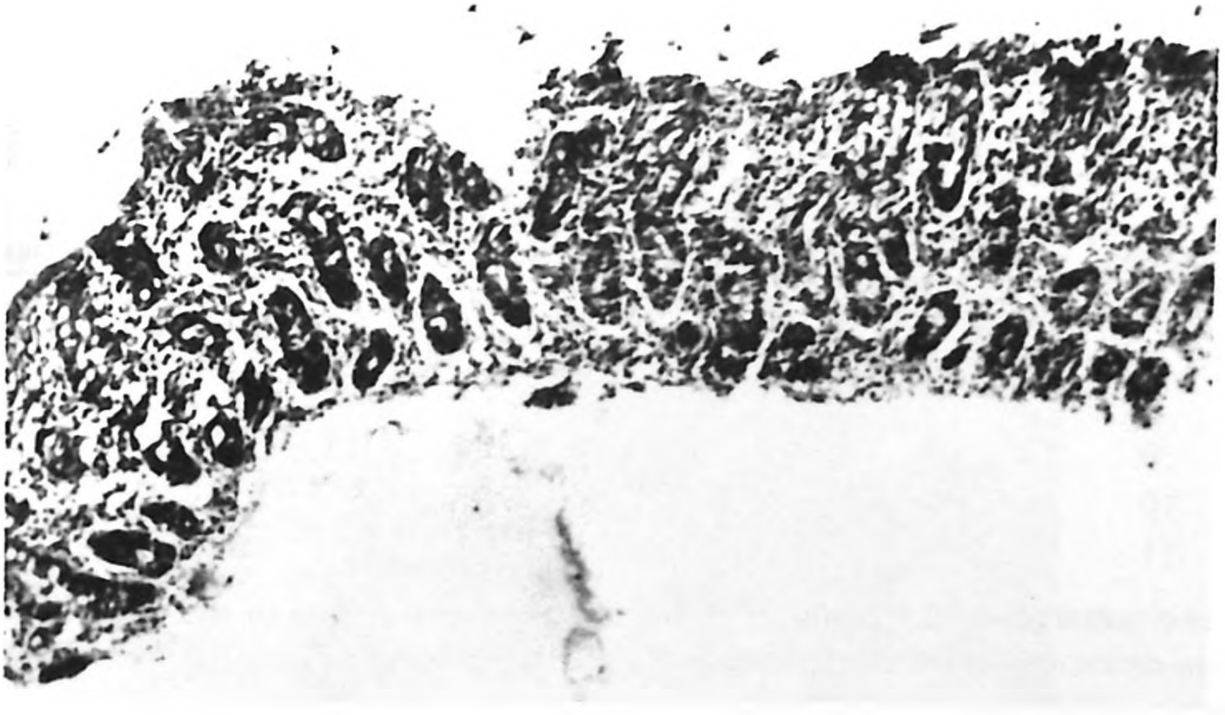

Fig. 2 (Case 9): Grade III villous damage: absence of villous structure, flat intestinal mucosa, surface epithelium irregular and cubic, and mucosa consisting only of crypt zone (H+Ex80).

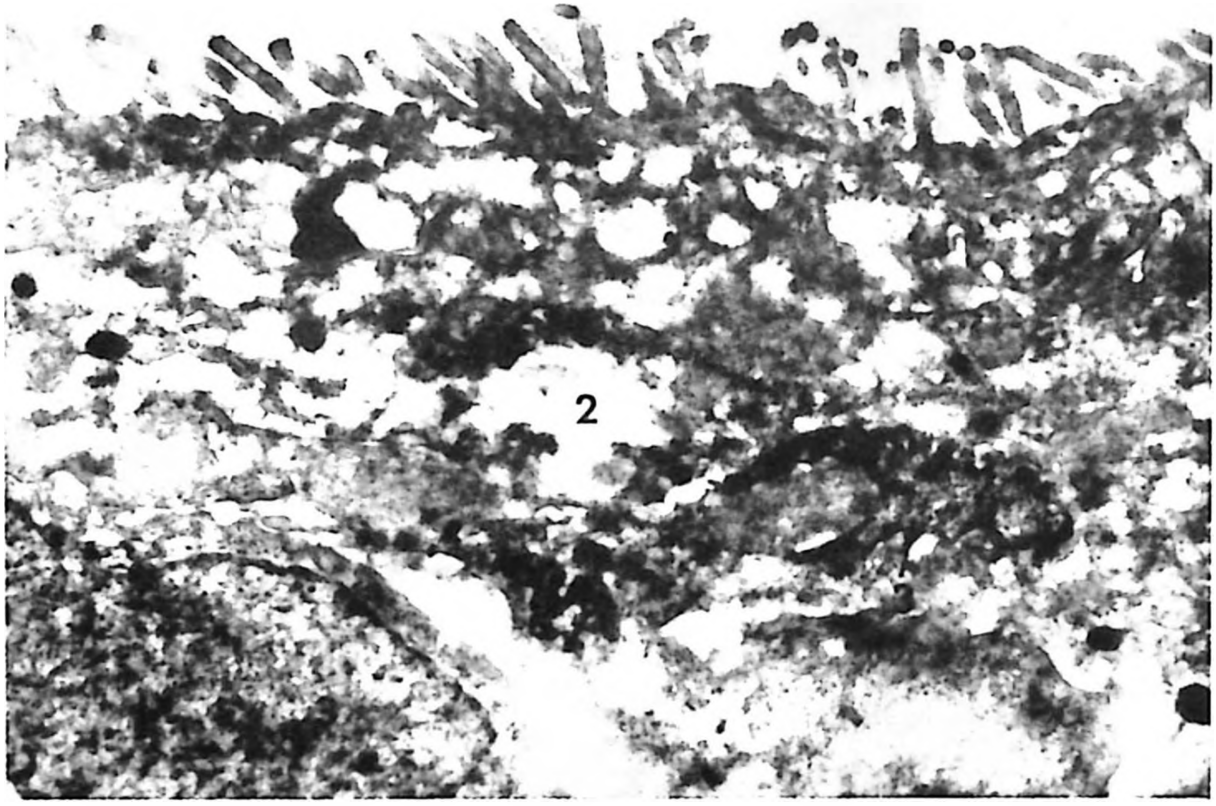

Fig. 3 (Case 9): 1-microvilli, 2-vacuole, 3-nucleus (x10,200). Microvilli are rarefied and irregular, filaments are not seen manifest, terminal web and surface coating are not visible. Nucleus of the epithelial cell on the right is picnotic.

TABLE III: Electron Microscopic Findings of Surface Epithelium in Patients With Iron Deficiency

Case No.	Microvilli lesion	Mitochondrial changes	Increase in lysosomes
8	–	+	+
7	–	–	–
8	+	+	+
9	+	+	+
10	–	–	–
11	–	+	+

in two patients (Cases 8 and 9). In these specimens cristae of the mitochondria were destroyed and the matrices were diluted, in other words, the mitochondria were swollen. In one of these two specimens, the disconnection of epithelial cells attracted our attention (Figs. 4 - 6). In these specimens, an increase of lysosomes was also observed.

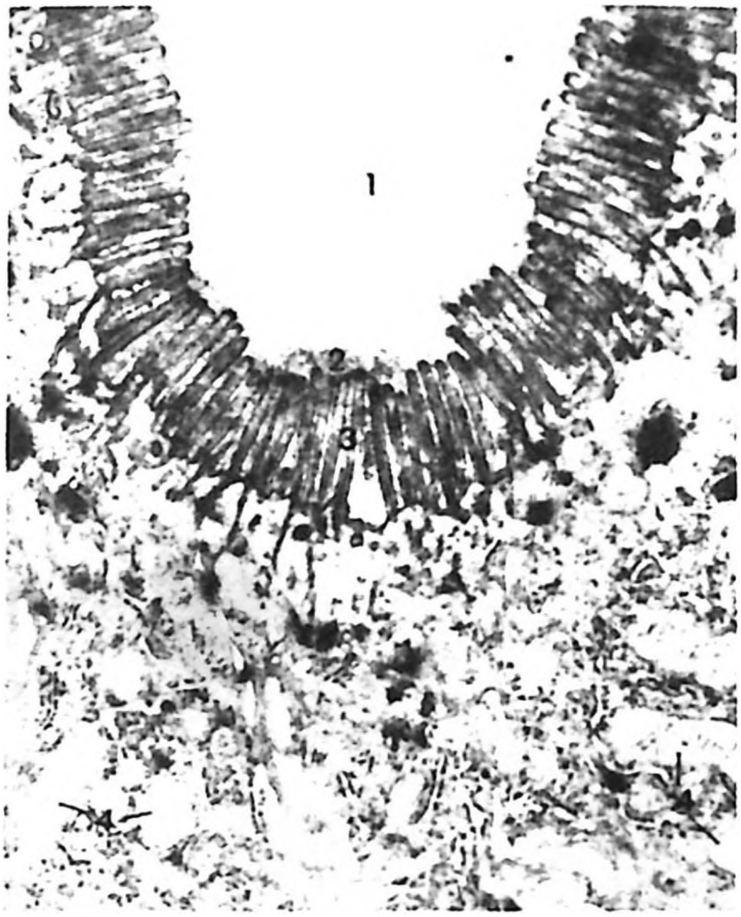

Fig. 4 (Case 9): 1-lumen, 2-surface coating 3-microvilli, 4-mitochondria (x10,200). This electron micrograph was taken from the crypt zone. Surface coating is normal, cristae of the mitochondria are destroyed and the matrix is diluted (mild swelling).

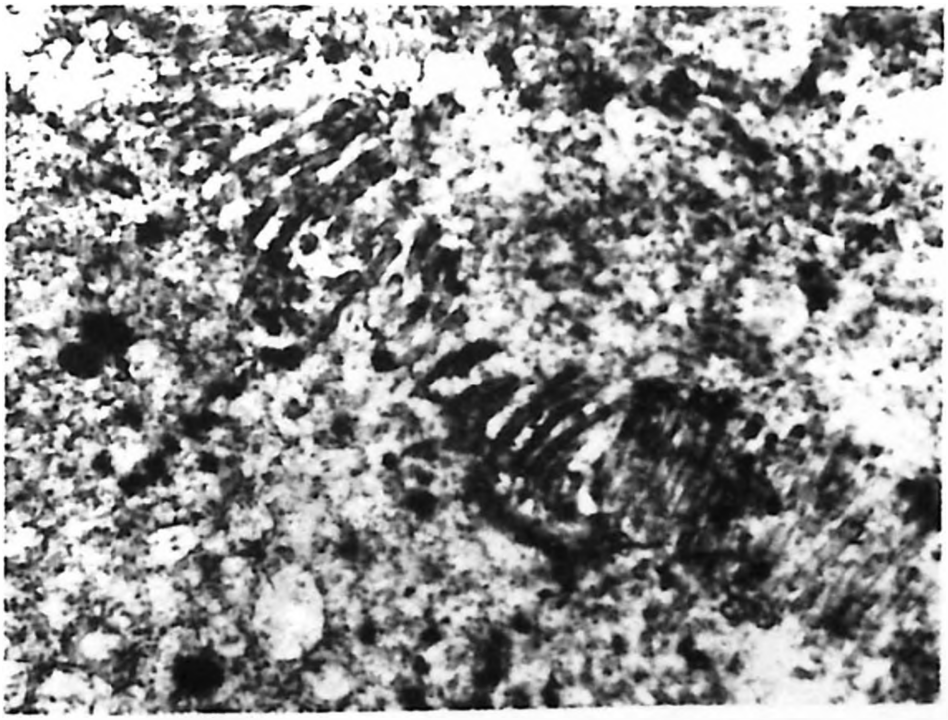

Fig. 5 (Case 8): 1-lumen, 2-microvilli, 3-lysosome, 4-mitochondrion (x10,200). Loss of microvilli and swelling of mitochondrion.

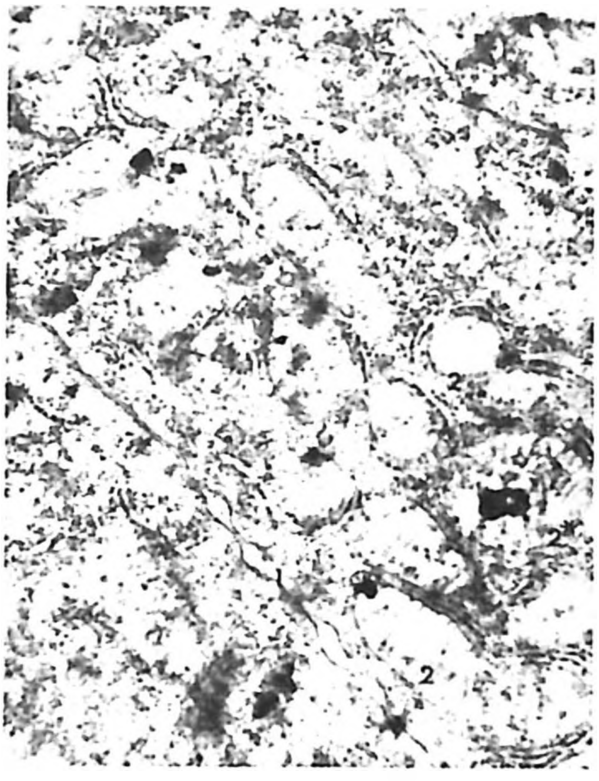

Fig. 6 (Case 8): 1-cell border, 2-mitochondria (x1,000). Disconnection between cells is seen. There is degeneration in a mitochondrion (mitochondrion-lysosome transformation). Mitochondria are swollen.

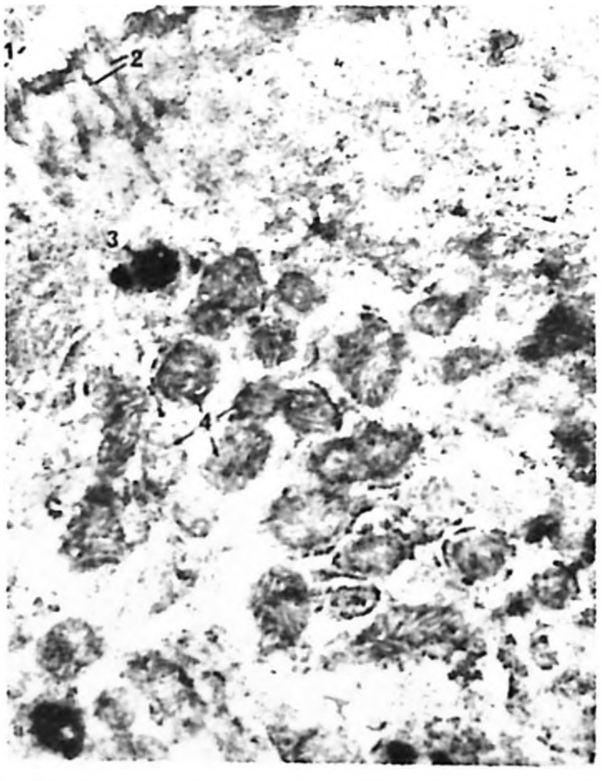

Fig. 7 (Case 6): 1-microvilli, 2-filament, 3-lysosome,4-mitochondria (x16,000). Mitochondria are small, cristae are prominent.

In the other two specimens (Cases 6 and 11), the mitochondria were small, the cristae prominent, and there was a slight increase in the density of the matrices (Fig. 7). In one of these specimens, the mitochondria had a normal appearance after therapy (Fig. 8). Ultrastructural findings are summarized in Table III.

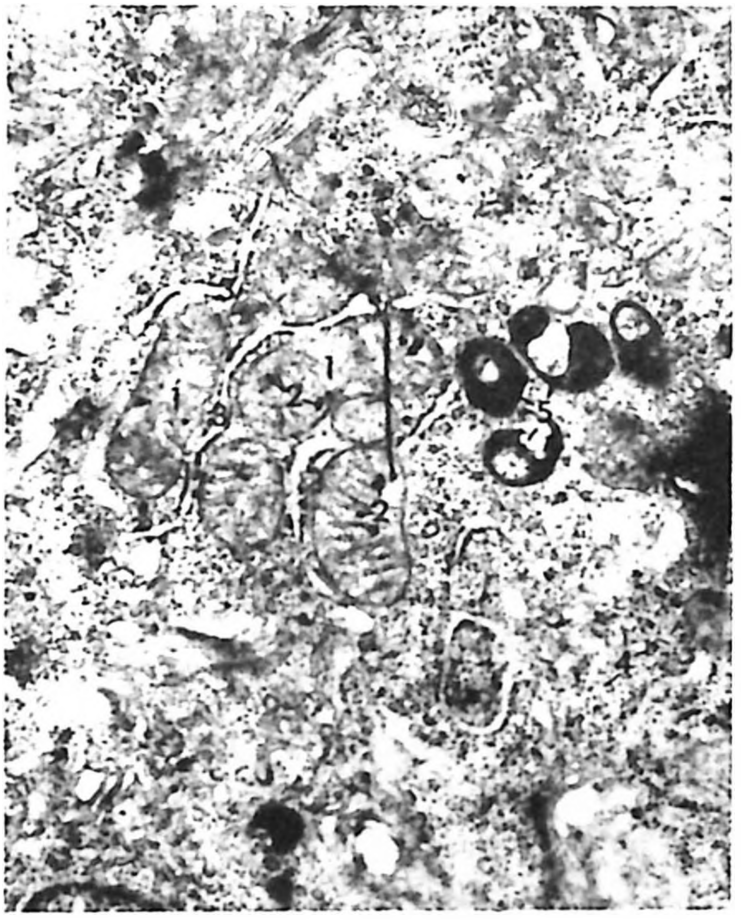

Fig. 8 (Case 6): 1-mitochondria, 2-intramitochondrial granules,3-granular endoplasmic reticulum, 4-free ribosomes, 5-lysosomes (x16,000). After therapy the mitochondria appear normal.

Intestinal function (Table IV): D-xylose absorption was impaired in only two patients (Cases 8 and 9), according to the one-hour blood d-xylose levels. Flat iron absorption curves were obtained in three cases (Cases 4, 8 and 9). The curves were elevated in the other patients. The maximal rise in serum iron as graphed on the elevated absorption curves ranged from 150 to 437 μg/dl.

Discussion

The incidence of intestinal mucosal changes, occurring as a result of iron deficiency anemia, has been reported to be 73 and 64 percent by certain investigators[4, 5]. In our study, light microscopic examination revealed intestinal mucosal changes in the specimens of 55 percent of the patients. Similar changes have also been observed in patients with iron deficiency and geophagia[12-15].

TABLE IV: Intestinal Function and Structure in Iron Deficiency

	D-xylose Test		Iron Absorption Test			
Case No.	1. Hour Blood d-xylose level	Grade of Villous Change	Fasting	1. Hour	3. Hours	Maximum Rise Over Baseline Values
	mg x/dl		μ g/dl			
1	75*	I				
2	44*	I	23.3	118.3	341.6	318.3
3	56*	0	57	285	392	335
4	53*	II	3.2	33.3	85	81.3
5	68*	I	27	300	515	215
6	38.3**	0	32	151	256	224
7	—	0	30	216	467	437
8	18.3**	II	34	44	94	60
9	13**	III	38	48	77	39
10	22.6**	0	33	54	183	150
11	25.9**	0	38	241	333	295

* With 0.5 g/kg d-xylose
** With 5 g d-xylose

There are some investigators who have reported no intestinal mucosal lesions in children with iron deficiency but these workers examined the specimens of only three patients[6]. Another group of investigators, who found Grade I and II abnormalities in a minority of their patients, believed these changes to be insignificant because of the geographical area where the study was carried out[7].

In our group of patients, in which intestinal mucosal lesions were detected by light microscopy, the younger patients seemed to have less severe abnormalities than the older ones, possibly due to the fact that the former group had the disease for a shorter period of time than the latter group. The intestinal mucosal changes seen in one of our patients, who was ten years old, were similar to those seen in patients with celiac disease, as reported by Guha et al[4]. Three of the patients with a history of pica had mucosal changes, but these changes were also observed in the patients without a history of pica. There was no significant correlation between pica and the presence of mucosal damage, but pica seems to have a deteriorative effect.

In some of our patients, normal intestinal mucosa was observed by light microscopy, which had no correlation whatsoever with age. When we compared

the group with intestinal mucosal lesions to the group without intestinal mucosal lesions, we found that there were no differences in hemoglobin levels, serum iron concentrations or transferrin saturation indices to explain the differences between the two groups, There was also no correlation between these parameters and severity of the intestinal mucosal lesion. These results are concordant with the observations of other investigators[4, 5].

Several authors have demonstrated that the abnormalities regress or the mucosa returns to normal with iron therapy, even in patients with very severe lesions[4, 5-13], which suggests that iron deficiency is responsible for pathological changes. On the other hand, Arcasoy et al[15] are of the opinion that chronic zinc deficiency caused more morphological abnormalities than iron in their patients with geophagia and iron deficiency, since these changes improved markedly following short-term therapy with zinc alone. One of our two cases, with microvillus lesions, practiced geophagia. The biopsy specimens of these patients revealed Grades II and III villous changes and mitochondrial changes such as swelling and an increase in the number of lysosomes. Such changes in the mitochondria have been observed in hepatic and myocardial mitochondria of iron deficient rats. Mitochondrial swelling, and in some cases, even rupture of the outer membrane of the mitochondria have been seen in peripheral blood lymphocytes in iron deficiency anemia[16].

The electron transport pathway in the mitochondria contains a variety of iron compounds that generate energy. In our patients, the lack of iron compounds along this pathway might have caused the chemical derangement to occur in the mitochondria leading to alterations in membrane permeability which caused the mitochondrial swelling. The mitochondrial changes, increased activity in the crypts, and the villous damage we observed suggest that due to the failure of the system to produce energy, the life of the surface epithelium was shortened and the cells were shed more rapidly than normal, and at a higher rate than repair. The increase in the number of lysosomes might be a sign of this rapid shedding since lysosomes may increase in frequency during the stress of rapid proliferation[17]. Since the gut is a major immunological organ, an increase in lymphoplasmocytic infiltration might be an expected finding in the presence of villous damage[18]. In some of our patients with normal intestinal mucosas, we also observed mitochondrial changes by light microscopy. Since the importance of the mitochondria in cell metabolism is known, mucosal lesions might develop if these patients are not treated. In one of these patients, the mitochondria appeared normal after therapy, which indicates that iron deficiency is responsible for mitochondrial changes.

Recently, Arcasoy et al[19] reported, in a study of patients with Prasad's syndrome, that not many structural alterations were detected on the surface epithelium.

However, in some cells, the microvilli were observed to have formed bunches of varying numbers of microvilli, some of which were branching. In the said study, ultrastructral alterations were mainly observed in Paneth's cells, and as a possible cause of these changes, zinc deficiency was mentioned.

Several studies have shown that there is malabsorption of d-xylose in iron deficiency, and the incidences reported were 13 percent, 65 percent, 18 percent, and 43 percent, respectively[1, 4, 5, 7]. In our patients,the incidence of d-xylose malabsorption was 15 percent. Intestinal mucosal damage and alterations in the structure of the microvilli were present in our patients with d-xylose malabsorption. Changes in the microvilli are believed to markedly reduce the absorptive surface[20]. When the absorption mechanism of d-xylose is taken into consideration, impaired d-xylose absorption in our patients might have been caused by the alterations in the structure of the villi and microvilli. D-xylose absorption was normal in one of our patients (Case 4) with the same grade villous change. We could not examine the microvilli in this patient but milder changes in the surface epithelium, which were detected by light microscopy, attracted our attention.

Schulz et al[21, 22] reported that children with iron deficiency anemia absorb food iron and a ferrous salt more efficiently than do normal children. Gross et al[23] showed that children without iron deficiency demonstrated flat absorption curves. They also showed that iron absorption curves were elevated in most iron deficient children but that some of their patients demonstrated impaired iron absorption. We observed elevated absorption curves in most of our patients. Our three patients with flat absorption curves had either Grade II or Grade III villous changes. Ultrastructural examination carried out in two of these patients revealed microvilli lesions and mitochondrial swelling. Mucosal uptake of iron, the first step in iron absorption, occurs rapidly on the brush border of the mucosal cell which consists of microvilli[24] and where specific receptors are thought to exist[25]. The changes in the microvilli that we observed might have impaired the absorption of iron by affecting these receptors. On the other hand, the appearance of the mitochondria suggests that the mucosal uptake and the transfer of iron from the mucosal cells to the lamina propria might be limited by the failure of sufficient energy production, as both steps probably represent energy dependent active transport processes[25]. In our third patient (Case 4), the fact that the maximal increase in iron concentration was greater than in the other two patients is concordant with the milder changes of surface epithelium and normal d-xylose absorption. In previous studies it was reported that d-xylose and iron malabsorption disappeared with iron therapy and, it was therefore concluded, that iron deficiency resulted in functional changes[4, 5, 23].

The elevated absorption curves observed in our patients with mitochondrial changes, other than the swelling, suggest that these mitochondria are still able to produce sufficient energy.

Kimber and et al[8] found that cytochrome oxidase activity was reduced in the intestinal mucosa of iron-deficient dogs and they believed that a decrease in iron-containing or iron-dependent enzyme systems in the mucosa of iron-deficient subjects might lead to abnormalities of cellular metabolism and a secondary state of malabsorption. This conclusion is similar to that of Dallman et al[9].

In our patients, no relationship was found between certain parameters of iron deficiency, and intestinal functional and morphological abnormalities, but morphological and functional changes were correlated. In one patient, the normal appearance of the mitochondria after therapy and the mitochondrial changes we observed suggest that impairment of cell metabolism due to a decrease of enzymes which contain iron or heme or require iron or heme as a cofactor, as pointed out by other authors[8, 9], might be responsible for the morphological and functional changes. We believe enzyme determinations and examination of ultrastructure in a greater number of iron deficient patients are needed to better understand the pathogenesis of these changes.

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